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CONTENTS FOR FEBRUARY, 1917

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SOIL SCIENCE

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THE ORGANIC MATTER OF THE SOIL: IV. SOME DATA ON HUMUS-PHOSPHORIC ACID[1]

By

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INTRODUCTION

Grandeau (10) in 1872 developed a method for the separation of the *matière noire* from the soil by ammonia extraction. According to Grandeau the material so separated may contain a very considerable proportion of ash, and this ash he regarded as an integral part of the humus. He ascribed the remarkable fertility of the black soils of Russia to the high proportion of ash in the humus (2.1 per cent humus and 2.1 per cent humus ash), this ash consisting largely of silica, phosphoric acid, lime, iron and magnesia.

Grandeau's views have been widely accepted in America, *cf.* Hilgard (11), Ladd (13), Snyder (18, 19, 20, 21), etc., the only noteworthy difference being that these authors do not report the humus ash as an integral part of the humus but call only the volatile portion "humus." However, they held that the humus is, at least in part, combined in the soil with inorganic substances; these compounds were termed "humates" and to their abundance and continued production was ascribed an important part in the maintenance of soil fertility.

Nefedov (14) extracted the humus from a Chernozem soil of Russia by means of ammonia and after mixing the humus with lime and crushed quartz tested its ability to nourish plants. He found that the media so formed disturbed the regular development of plant growth (barley and peas), intensifying the formation of shoots and rendering impossible the formation of reproductive organs.

Snyder (18) carried out a similar experiment and succeeded in securing fertile seeds from oats grown on a humus-gypsum-quartz sand medium. Snyder does not regard all of the mineral matter extracted with the humus as in chemical combination with the humus, but he does regard [20 (b), p. 31] the mineral matter in that portion which is precipitated by acids as in organic combinations.

[1] Received for publication November 18, 1916.

[2] The work embodied in this paper was carried out in the Division of Soils when the authors were Associate Soils Chemist and Student, respectively.

R. Stewart (23) attempted to determine how much of the soil phosphorus was in organic combination by first extracting a soil with 12 per cent HCl and then dividing this extracted soil into two samples one of which he extracted with 12 per cent HCl and the other with 4 per cent ammonia. The excess of P_2O_5 dissolved by the ammonia over that dissolved by the HCl he regarded as of organic origin. J. Stewart (22) believes that all of the phosphorus of the *matière noire* is derived from organic sources, but that a part is, however, present in inorganic form inasmuch as hydrolysis of the organic phosphorus compounds occurs during the extraction with alkali.

Fraps (6) shows that there are certain objections to R. Stewart's (23) analytical procedure and demonstrates that certain of the phosphate minerals are more soluble in 4 per cent ammonia than in 1 per cent (or even 12 per cent) HCl. He therefore argues that the humus-phosphoric acid contains both inorganic and organic phosphorus, but that in all probability the inorganic phosphorus predominates.

Potter and Benton (15) have very recently published a preliminary paper in which they suggest a method for the separation of the organic and inorganic phosphorus in a humus extract. In eight soils they find from 68.7 to 87.3 per cent of the phosphorus in the ammonia extract to be in organic combination. Their method consists of precipitating both inorganic phosphates and organic phosphorus-containing compounds from an alkaline solution with magnesia mixture, dissolving the inorganic magnesium ammonium phosphate with dilute acid and determining it in the resulting acid solution. The difference between the total P_2O_5 in the alkali extract and the "inorganic" P_2O_5 is regarded as organic. The possibility still remains, however, that a portion of the "organic" P_2O_5 is derived from inorganic P_2O_5 set free by the 1 per cent HCl leachings and adsorbed by the colloidal organic matter. Also, any phosphoric acid contained in colloidal mineral materials (clay, etc.) would appear as "organic" P_2O_5. That a certain amount of clay is present in the procedure is, we believe, without question; the high percentage of ash in the soil pigment preparations (8) shows that the colloidal organic matter of the soil and colloidal clay separate together from solution. Alway *et al* (2) point out that the humus ash varies from method to method, and conclude, probably correctly, that "the greater portion of the humus ash obtained by any of the methods is not an essential part of the humus." Until we can prepare a "humus" which does not contain a large percentage of ash consisting in the main of silica [*cf.* Snyder (19, p. 741)] there would appear to be no definite decision possible regarding the actual proportion of the phosphorus which is in organic combination. It will be necessary first to devise some method which will make allowance for the presence of clay and complex inorganic

phosphorus compounds which may be *adsorbed* by the colloidal organic matter and rendered relatively insoluble in acids.

In so far as we are aware, the only direct evidence that phosphorus occurs in the soil in organic combinations is furnished by Aso (5) who obtained ether-alcohol soluble phosphorus from soil, Stoklasa (24) who obtained evidences of lecithin and Shorey (16, 17) who isolated nucleic acids. In all probability a certain part, if not all of these compounds, were products extracted from living bacteria or protozoa. While phytin is present to a very considerable amount in seeds of certain plants, no evidence has been presented to show that it is present in the roots and stems, the materials from which most of the soil organic matter is derived. It is certain that potassium hydrogen phosphate is present in many vegetable saps so that Snyder's [20 (b), p. 15] assumption that all of the phosphoric acid present in vegetable material would eventually be converted into humus phosphoric acid does not necessarily hold true.

The present authors have but little to add to the subject of humus-phosphoric acid. In the preceding papers of this series (7, 8, 9) a number of "humus" determinations have appeared. In all of these instances the phosphoric acid in the "humus ash" has been determined and the data are here presented, more with the idea of adding to the data on this series of soils than with the idea that any of the conclusions are fundamentally new. It does appear, however, in view of the wide range of opinions on the value of humus-phosphoric acid determinations that these observations are worth recording.

Experimental

The description of the soils used, the methods of treatment in preparing the "humus" extracts and the method of estimating "humus" and "humus ash" have been fully presented in the preceding papers (7, 8, 9) so that it is unnecessary to repeat them here.

The Determination of Phosphoric Acid in the Humus Ash. The ash, remaining after the ignition of the humus, was treated with 10 to 15 c.c. of aqua regia in the quartz dish in which the ignition was made and digested on a steam bath until all iron had been dissolved and only white particles of silica remained. The solution was then evaporated to dryness, 10 c.c. of nitric acid added and evaporated to complete dryness and the evaporation with nitric acid again repeated in order to convert all chlorides into nitrates. The dry residue was then moistened with concentrated nitric acid and digested on the steam bath until all iron was in solution, after which the solution was diluted, filtered from the white insoluble silica and the phosphorus in the filtrate determined gravimetrically as magnesium pyrophosphate after precipitating with ammonium nitrate and ammonium molybdate in the usual manner.

The phosphorus extracted by 1 per cent HCl was estimated by evaporating the leachings to dryness, incinerating, and proceeding exactly as above.

The analytical data, together with certain ratios which have been calculated, appear in Tables I to IV. All of the percentages are the average of closely agreeing duplicate determinations.

TABLE I

SHOWING THE RELATIONSHIPS BETWEEN HUMUS, HUMUS ASH AND HUMUS-PHOSPHORIC ACID EXTRACTED FROM THE UNLEACHED SOILS OR VEGETABLE MATERIALS BY 4 PER CENT NH_4OH, $B(NH_4OH)$

Soil or Vegetable Material Extracted	Per cent Humus in Soil	Per cent Humus Ash in Soil	Ratio of Humus to Humus Ash	Per cent Humus Phosphoric Acid	Per cent P_2O_5 in Humus	Per cent P_2O_5 in Humus Ash
Fargo clay loam	0.95	0.46	2.1	0.013	1.370	2.83
Forest-covered loess	1.16	0.25	4.6	0.049	4.220	19.60
Berkeley adobe	1.24	0.33	3.7	0.039	3.150	11.82
Prairie-covered loess	1.42	0.33	4.3	0.022	1.550	6.67
Marshall silt loam	1.49	0.39	3.8	0.039	2.620	10.00
Hempstead silt loam	2.00	0.62	3.2	0.056	2.800	9.03
Carrington silt loam	2.86	0.34	8.4	0.045	1.570	13.24
Fargo silt loam	3.65	0.46	7.9	0.045	1.230	9.78
Average for soils	1.85	0.40	4.7	0.038	2.310	10.37
Muck	5.82	1.88	3.1	0.076	1.310	4.04
Black peat	12.97	1.13	11.5	0.074	0.570	6.55
Brown peat	35.29	2.25	15.7	0.181	0.513	8.04
Sphagnum-covered peat	34.40	1.79	19.2	0.150	0.436	8.38
Average for peats	22.12	1.76	12.4	0.120	0.707	6.75
Oak leaves	35.94	2.23	16.1	0.097	0.270	4.35
Oats	36.09	4.62	7.8	0.482	1.340	10.43
Grass from brown peat bog	36.46	4.45	8.2	0.199	0.546	4.47
Sweet fern leaves	41.70	2.46	17.0	0.101	0.242	4.10
Alfalfa hay	51.73	6.06	8.5	0.240	0.463	3.96
Average for vegetable materials	40.38	3.96	11.5	0.224	0.572	5.46
Soil A—1915	0.60	0.29	2.1	0.016	2.660	5.51
Soil A—1916	0.56	0.28	2.0	0.012	2.140	4.29
Soil B—1915	1.05	0.37	2.8	0.013	1.240	3.51
Soil B—1916	1.07	0.28	3.8	0.008	0.747	2.86
Soil C—1915	0.75	0.28	2.7	0.016	2.130	5.71
Soil C—1916	0.79	0.25	3.2	0.014	1.770	5.60
Soil D—1915	1.55	0.35	4.4	0.016	1.030	4.57
Soil D—1916	0.61	0.28	2.2	0.025	4.100	8.92

Discussion

It is of interest to note certain of the relationships between the percentages of phosphoric acid extracted by the three treatments. Potter and Benton (15, p. 295) express the belief that the phosphorus extracted from a soil by dilute acid is entirely inorganic in nature. It appears very probable, however, that if phytin were present in a soil the 1 per cent HCl would remove the calcium or magnesium radicle,

thus liberating the free acid which would be readily water-soluble and would appear in the HCl leachings. J. Stewart (22, p. 284) attempted to determine humus and ammonia-soluble P_2O_5 in a soil *without a preliminary leaching with acid* and reached the conclusion that *soil*

TABLE II

SHOWING THE RELATIONSHIPS BETWEEN HUMUS, HUMUS ASH AND HUMUS-PHOSPHORIC ACID EXTRACTED FROM THE SOILS OR VEGETABLE MATERIALS BY 4 PER CENT NH_4OH AFTER A PREVIOUS EXTRACTION WITH 1 PER CENT HCl, AB(NH_4OH)

Soil or Vegetable Material Extracted	Per cent Humus in Soil	Per cent Humus Ash in Soil	Ratio of Humus to Humus Ash	Per cent Humus Phosphoric Acid	Per cent P_2O_5 in Humus	Per cent P_2O_5 in Humus Ash
Forest-covered loess	1.80	0.45	4.0	0.056	3.110	12.440
Berkeley adobe	2.39	0.48	5.0	0.056	2.340	11.660
Fargo clay loam	2.66	0.56	4.8	0.020	0.750	3.570
Marshall silt loam	2.85	0.37	7.7	0.063	2.210	17.020
Prairie-covered loess	3.40	0.42	8.1	0.080	2.350	19.040
Hempstead silt loam	3.61	0.52	7.0	0.095	2.630	18.260
Carrington silt loam	4.95	0.40	12.4	0.113	2.280	28.250
Fargo silt loam	9.91	0.72	13.7	0.141	1.430	19.580
Average for soils	3.95	0.49	7.8	0.078	2.140	16.230
Muck	7.14	0.30	23.8	0.104	1.450	34.670
Black peat	28.71	1.74	16.5	0.205	0.710	11.780
Sphagnum-covered peat	32.91	1.47	22.4	0.127	0.386	8.640
Brown peat	39.22	1.87	20.9	0.185	0.472	9.890
Average for peats	26.99	1.35	20.9	0.155	0.755	16.250
Oats	26.65	2.63	10.1	0.134	0.503	5.100
Alfalfa hay	29.40	0.84	35.0	0.229	0.778	27.260
Oak leaves	29.81	2.34	12.7	0.009	0.030	0.385
Sweet fern leaves	31.81	3.20	9.9	0.060	0.188	1.870
Grass from brown peat bog	31.95	2.44	13.1	0.111	0.346	4.550
Average for vegetable materials	29.92	2.29	16.2	0.109	0.369	7.830
Soil A—1915	0.75	0.39	1.9	0.052	6.930	13.330
Soil A—1916	0.67	0.26	2.6	0.039	5.820	15.000
Soil B—1915	1.17	0.41	2.9	0.042	3.590	10.240
Soil B—1916	0.83	0.31	2.7	0.041	4.940	13.220
Soil C—1915	0.78	0.39	2.0	0.034	4.360	8.710
Soil C—1916	0.73	0.36	2.0	0.037	5.070	10.270
Soil D—1915	1.26	0.31	4.1	0.033	2.620	10.640
Soil D—1916	0.84	0.32	2.6	0.036	4.280	11.250
Subsoil—1915	0.58	0.42	1.4	0.047	8.100	11.190

phosphorus is insoluble in 4 per cent NH_4OH unless the soil has been previously leached with acid. In direct opposition to this latter finding are the determinations reported in Table I. It will be observed that in three of the eight soil types *more phosphorus was dissolved from the unleached soil by 4 per cent NH_4OH than by 1 per cent HCl* (Table III) and in only *one* of the soils is the quantity of P_2O_5 in the HCl leachings markedly greater than that in the "unleached humus," B(NH_4OH). When we contrast the P_2O_5 in the "leached humus,"

AB(NH_4OH) (Table II) with that in the HCl leachings, much the same finding is apparent, *i. e.*, the ammonia extracts more P_2O_5 from all of the soils *with one exception* than does the acid. There does not, however, appear to be any relationship between the quantities of P_2O_5 extracted by

TABLE III

THE PERCENTAGE OF PHOSPHORIC ACID PRESENT IN THE 1 PER CENT HCl LEACHINGS FROM THE DIFFERENT SOILS OR VEGETABLE MATERIALS AND CERTAIN RATIOS BETWEEN THIS PHOSPHORIC ACID CONTENT AND THE OTHER ANALYSES

Soil or Vegetable Material Extracted	P_2O_5 in HCl Leachings	Ratio of P_2O_5 in HCl to P_2O_5 in "Unleached Humus" B(NH_4OH)	Ratio of P_2O_5 in HCl to P_2O_5 in "Leached Humus" AB(NH_4OH)	Ratio of P_2O_5 in "Leached Humus" to P_2O_5 in "Unleached Humus"
Carrington silt loam	0.014	0.31	0.12	2.51
Hempstead silt loam	0.016	0.29	0.17	1.70
Forest-covered loess	0.019	0.38	0.34	1.14
Prairie-covered loess	0.023	1.05	0.29	3.64
Berkeley adobe	0.044	1.13	0.79	1.44
Marshall silt loam	0.042	1.08	0.67	1.62
Fargo silt loam	0.053	1.17	0.38	3.13
Fargo clay loam	0.064	4.92	3.20	1.54
Average for soils	0.034	1.29	0.75	2.09
Brown peat	0.035	0.19	0.19	1.02
Muck	0.055	0.72	0.53	1.37
Sphagnum-covered peat	0.087	0.58	0.69	0.85
Black peat	0.135	1.82	0.66	2.77
Average for peats	0.078	0.83	0.52	1.50
Sweet fern leaves	0.083	0.82	1.38	0.59
Oak leaves	0.116	1.20	12.89	0.09
Grass from brown peat bog	0.144	0.72	1.30	0.56
Alfalfa hay	0.402	1.67	1.76	0.90
Oats	1.060	2.20	7.91	0.28
Average for vegetable materials	0.361	1.32	5.05	0.48
Soil A—1915	0.044	2.75	0.85	3.25
Soil A—1916	0.049	4.08	1.26	3.25
Soil B—1915	0.047	3.62	1.12	3.23
Soil B—1916	0.054	6.75	1.32	5.12
Soil C—1915	0.055	3.44	1.62	2.13
Soil C—1916	0.063	4.50	1.70	2.64
Soil D—1915	0.070	4.37	2.12	2.06
Soil D—1916	0.068	2.72	1.89	1.44
Subsoil—1915	0.050	....	1.06	....

the ammonia under the two different conditions, and there should be such a relationship if only organic P_2O_5 were extracted in both instances. It is likewise extremely interesting to note that *the Fargo clay loam, the famous soil type of the valley of the Red River of the North, contains by far the least humus-phosphoric acid of all the soil types examined.* How is this reconciled with the theory that humus-phosphoric acid is

an indication of the availability of soil phosphorus, and is a necessary factor in soil fertility?

"Leached humus" and humus-phosphoric acid. In Table II the eight soil types have been arranged in order of the humus contents. As the humus content increases there is a marked tendency of the ratio of

TABLE IV

SHOWING THE RELATIONSHIPS BETWEEN THE TOTAL NITROGEN OF THE SOIL AND THE PHOSPHORIC ACID IN THE HUMUS ASHES AND THE HYDROCHLORIC ACID LEACHINGS

Soil or Vegetable Material Extracted	Per cent Total N. in Soil	Ratio of Total N. to P_2O_5 in the "Unleached Humus" $B(NH_4OH)$	Ratio of Total N. to P_2O_5 in the "Leached Humus" $AB(NH_4OH)$	Ratio of Total N. to P_2O_5 in the
Forest-covered loess	0.128	2.6	2.3	6.7
Marshall silt loam	0.237	6.1	3.8	5.6
Berkeley adobe	0.239	6.1	4.3	5.4
Fargo clay loam	0.250	19.2	12.5	3.9
Hempstead silt loam	0.256	4.6	2.7	16.0
Prairie-covered loess	0.301	13.7	3.8	13.1
Carrington silt loam	0.371	8.2	3.3	26.5
Fargo silt loam	0.823	18.3	5.8	15.5
Average for soils	0.326	9.8	4.8	11.6
Muck	1.340	17.6	12.9	24.4
Sphagnum-covered peat	2.000	13.3	15.7	23.0
Brown peat	2.818	15.6	15.2	80.5
Black peat	2.940	39.7	14.3	21.7
Average for peats	2.275	21.5	14.5	37.4
Oak leaves	0.998	10.3	110.9	8.6
Grass from brown peat bog	1.164	5.8	10.5	8.1
Sweet fern leaves	1.585	15.7	26.4	19.1
Oats	2.181	4.5	16.3	2.1
Alfalfa hay	3.798	15.8	16.6	9.4
Average for vegetable materials	1.945	10.4	36.1	9.5
Soil A—1915	0.334	20.9	6.4	7.6
Soil A—1916	0.304	25.3	7.8	6.2
Soil B—1915	0.349	26.8	8.3	7.4
Soil B—1916	0.260	32.5	6.3	4.8
Soil C—1915	0.119	7.4	3.5	2.2
Soil C—1916	0.113	8.1	3.1	1.8
Soil D—1915	0.151	9.4	4.6	2.2
Soil D—1916	0.111	4.4	3.1	1.6
Original subsoil	0.050	...	1.1	1.0

humus to humus ash to increase, showing that the "ash" of the humus does not vary directly with the humus but lags behind, therefore indicating that the humus and ash may not be casually related. The percentage of P_2O_5 in the humus ash increases slightly, but probably not significantly, with the higher percentages of humus. The high percentage of P_2O_5 in the humus ash would, *per se,* seem to indicate that

a part of the phosphoric acid was of organic origin; however, the "subsoil-1915" (from the 3rd foot of the Hempstead silt loam) having a humus content of only 16 per cent of that of the surface soil shows a humus P_2O_5 percentage equal to one-half that of the surface soil. There is certainly no such difference in the P_2O_5 content of the organic materials at these different depths; in fact, the "humus" solution from the subsoil was practically colorless and only a few flecks separated when the solution was acidified, so that a content of 11.19 per cent of P_2O_5 in the humus ash must be regarded as largely of inorganic origin due probably to the phenomenon of *selective adsorption*. We know that P_2O_5 is adsorbed and retained by the soil colloids as well as bound chemically when a phosphate solution is percolated through a soil. Such a percolation actually takes place when a soil is leached with 1 per cent HCl and Fraps (6, p. 7) states that soils are able to remove phosphoric acid from acid solutions. May it not be that the colloidal material of the soil acts in a similar manner to that of the hydrous aluminum silicate prepared by Lloyd (12) which adsorbs alkaloids completely from an acid solution, liberating them again in an alkaline solution? Such a possibility for a selective adsorption of phosphoric acid appears very probable in view of the remarkable specificity of "Lloyd's reagent" and *all of the adsorbed phosphoric acid would appear in the humus ash as humus-phosphoric acid and would be counted as organic phosphoric acid in Potter and Benton's method* (15), inasmuch as the final extraction of the magnesium ammonium phosphate with dilute acid by this method would again produce an acid reaction and render conditions favorable for the adsorption again to take place. An average increase of the P_2O_5 content of the humus ash from 10.37 per cent for the "unleached humus" to 16.23 per cent for the "leached humus" in the eight soil types speaks strongly for such an adsorption due to leaching the soil with acid, and an increase of the same order holds true for the "artificial humus" set of soils. The apparent increase in the peats is due entirely to the muck which behaves like a mineral soil. There is no noticeable change in the case of the other three peats. Neither is there any noticeable difference in the case of the vegetable materials, if we except the alfalfa hay, and here again there is a ready explanation, *i. e.*, the humus P_2O_5 is actually greater in the unleached humus while the acid extraction has reduced the ash from 6.06 per cent to 0.84 per cent, thus making an enormous difference in the percentage of P_2O_5 in the ash.

Another indication of such an adsorption is found in the phosphoric acid content of the ash from the residual humus determinations of the experiment reported in the second paper of this series (8). In this instance a soil was leached with 1 per cent HCl, the leached soil extracted nine times with 4 per cent NaOH and then six times with

dilute (approximately 0.15 per cent concentration) NaOH, sulfuric acid added to acid reaction, the residual soil washed free of sulfates, dried and ground. "Humus" was determined on the resulting "residual soil from Pigment Solution 6," 1.54 per cent "humus" and 0.74 per cent of ash being obtained. This ash was analyzed and phosphoric acid equal to 0.028 per cent of the original soil found, or, in other words, this ash contained approximately 4 per cent of P_2O_5 after 15 preliminary alkaline and two preliminary acid extractions. No other conclusion seems possible but that this P_2O_5 represents colloidal or absorbed mineral material and the high ash content of the humus (32 per cent of the ammonia-soluble materials) bears out this contention. The "humus" from the "residual soil from NaOH Solution I" which had been extracted nine times with 4 per cent NH_4OH contained P_2O_5 equal to 0.06 per cent of the soil weight. *It thus would appear probable that adsorption of phosphoric acid from the HCl leachings by ammonia-soluble soil colloids is responsible for a considerable part of the P_2O_5 found in the ash of the "leached humus."* Such phosphoric acid would be recorded as of organic origin by either Stewart's (23) or Potter and Benton's (15) methods of analysis.

Ratios of the total nitrogen of the soil to the phosphoric acid extracted by the different treatments. Inasmuch as organic phosphoric acid is in all probability largely associated with nitrogenous compounds (*e. g.* lecithin, and nucleic acids), it was thought worth while to see if any relationship existed between the total nitrogen content of the soil or vegetable materials and the phosphoric acid extracted by the different treatments. These ratios are given in Table IV. No relationship was found. It was not thought worth while to calculate similar ratios for organic carbon and phosphorus, inasmuch as a more or less constant relationship for carbon to nitrogen exists for the soils [*cf.* Alway and Vail (4) and Alway and McDole (3)]. Anyone wishing to calculate these ratios can readily find the necessary data in the preceding papers (7, 8, 9).

Ratio of humus to humus ash. In the case of the unleached samples of the eight soil types we find a minimum ratio of humus to humus ash of 2.1 with a maximum ratio of 8.4. These indicate a minimum of 10.6 and a maximum of 32.6 per cent of ash in the ammonia-soluble materials. Similarly we find in these samples a minimum of 6.7 per cent and a maximum of 20.0 per cent of ash in the ammonia-soluble materials from the "leached humus" data, while the percentage of ash in the case of the "subsoil-1915" reaches 42 per cent of the ammonia-soluble material. Some very interesting data on the percentage of ash in the humus as we progress downward in a soil column are furnished by Alway and Blish (1, p. 243-4). These authors determined humus

and humus ash in six sets of soil samples, each set consisting of samples from each foot section of the first six feet of soil. The *average* of all of these determinations (each of which is already the average of 10 individual determinations, so that each percentage here given represents the mean value for 60 determinations) are given in Table V together with the percentage of ash in the ammonia-soluble materials. The data presented need no discussion. It is easily seen that the humus bears no relationship to the humus ash, for while the humus content falls the ash remains practically stationary or even rises slightly. It is unfortunate that we do not have data showing the percentage of phosphoric acid in this "humus ash." Obviously these high percentages of ash are largely derived from the inorganic soil particles and bear no relationship to the dissolved organic material. Why then should the phosphoric acid in "humus ash" be considered as largely of organic origin? The above reasoning applies with equal force to determinations of humus-potash and it appears extremely improbable that organically-bound potash is present in the soil in any appreciable quantity.

TABLE V

AVERAGES FOR HUMUS, HUMUS ASH AND THE PERCENTAGE OF ASH IN THE AMMONIA-SOLUBLE MATERIALS FOR 1 TO 6 FOOT SECTIONS IN A VERY UNIFORM SOIL TYPE (LOESS): DATA OF ALWAY AND BLISH (1)

Depth Foot	Average Per cent Humus	Average Per cent Humus Ash	Average Per cent Ash in Ammonia-Soluble Materials
1	1.63	0.30	15.5
2	0.88	0.26	22.8
3	0.41	0.31	43.0
4	0.29	0.36	55.4
5	0.24	0.39	61.9
6	0.22	0.43	66.1
Average	0.61	0.34	35.8

Do "humifying" organic substances combine with soil phosphoric acid to form organo-phosphoric acid combinations? Snyder [20 (b)] presented some data in support of the theory that organic substances undergo a specific humification in the soil and that the humus so formed combines with soil phosphoric acid to form humus-phosphoric acid which he regards as more readily available for plant use than the phosphoric acid of mineral phosphates. Some fallacies in the humification theory have been pointed out in the preceding paper (9). Likewise the authors have in this paper (see above) referred to the fact that potassium hydrogen phosphate is present in the saps of many plants so that the assumption (20, p. 15) that all of the phosphoric acid present in vegetable materials would eventually be converted into humus-phosphoric acid does not necessarily hold true.

In the final series of soils in Tables I to IV are presented the data on the humus-phosphoric acid for a series of experiments similar to that carried out by Snyder [20 (b)]. These data indicate that the "humification" of vegetable materials during an entire year has not increased the quantity of "humus-phosphoric acid" over that contained in the original subsoil.

Summary

Experimental data have been presented dealing with the phosphoric acid content of the ammonia extract of certain soils, peats and unchanged vegetable materials, both before and after leaching the samples with 1 per cent HCl; the phosphoric acid content of the HCl leachings is also considered. Certain ratios between these phosphoric acid percentages and the nitrogen, humus, and humus ash contents of the samples, are presented and discussed. The following conclusions have been reached:

1. Ammonium hydroxide in 4 per cent concentration will, in certain soil types, extract more P_2O_5 from the air-dry soil than will 1 per cent HCl, and in only one sample of eight different soil types did hydrochloric acid extract appreciably more P_2O_5 than did the ammonia.

2. There appears to be no relation between the amounts of P_2O_5 extracted by ammonia from the unleached soil and that extracted after leaching with 1 per cent HCl, although in both instances there is usually a greater quantity of P_2O_5 extracted by the ammonia than is extracted by the acid.

3. The amounts of humus-phosphoric acid present in the eight soil types studied when compared with the known fertility of these soil types do not support the theory that a high humus-phosphoric acid content is a necessary factor in soil fertility.

4. Probably the greater part of the phosphoric acid present in humus ash is inorganic in nature, being derived from colloidal clay and from phosphoric acid adsorbed by the colloids present in the ammonia solution. No method of analysis has as yet been proposed which will distinguish between such adsorbed P_2O_5 and organic P_2O_5.

5. There is no relationship detectable between the total nitrogen content of the soil and the P_2O_5 extracted by the different treatments.

6. The amount of "humus ash" present in an ammonia extract is extremely variable, even when the extractions have been carried out under indentical working conditions. A minimum of 6.7 per cent and a maximum of 32.6 per cent of ash were found in the "humus" extracted from the eight mineral soil types studied. Such a wide divergence can be accounted for only by the presence of considerable amounts of clay or adsorbed mineral materials.

7. The "humification" of vegetable materials in contact with a mineral soil for an entire year did not increase the humus-phosphoric acid over that contained in the original subsoil.

8. The phosphoric acid present in the ammonia extract of soils cannot be correlated either with the amount of organic matter present or with the known fertility of the soil type. Inasmuch as this P_2O_5 does not represent a definite chemical entity, there appears to be no valid reason why determinations of humus-phosphoric acid should be made.

9. It is pointed out that the conclusions drawn above in regard to colloidal adsorbtion of P_2O_5 apply with equal force to determinations of humus-potash, that in all probability organically-bound potash does not occur in the soil in appreciable amounts.

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THE WATER CONTENT OF THE SOIL AND THE COMPOSITION AND CONCENTRATION OF THE SOIL SOLUTION AS INDICATED BY THE FREEZING-POINT LOWERINGS OF THE ROOTS AND TOPS OF PLANTS[1]

ABSTRACT

By

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Introduction

Although certain lines of work presented in the following abstract are not complete it seems advisable at this time to offer the following report on some of the work now in progress.

There are on record results of many studies of the concentration of the cell sap of aerial portions of plants, that is, the leaves and stems, but there is available much less information with respect to the concentration of the root sap of plants. Moreover, certain investigators have contributed valuable data bearing upon the reaction of the plant to the water content of soils and the concentration of the soil solution. Regardless of the numerous researches, there is much to be accomplished, especially by improving methods and studying the concentration and composition of the soil solution, and the water content of soils as measured by the lowering of the freezing-point of the roots and aerial portions of plants.

This paper presents the principal results obtained from studies of the concentration, and composition of the soil solution, and the water content of soils and the rate of water movement in soils as measured by the freezing-point lowerings of the roots and leaves of plants. A number of soils and plants have been utilized in these researches. Moreover, the results presented include studies under both greenhouse and field conditions.

We previously reported (2) that the freezing-point method can be used to determine the concentration of the cell sap directly in the plant tissue. Inasmuch as the details of the method were not presented, it is well to consider them at this time. About 12 gm. of the material to be studied is placed in a freezing tube and slightly macerated by means of a

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stiff wire flattened and sharpened at one end. The Beckmann thermometer is inserted into the mass, care being taken to have the mecury bulb covered and the tissue gently pressed about it; the tube is then placed directly into the ice and salt mixture (the temperature of which should be —2° to —3° C.) and kept there until the contents have supercooled about one degree. Solidification is brought about by gently agitating the thermometer. Immediately after the mercury begins to rise the tube is removed from the ice and salt mixture and placed in the air bath, and the reading taken when the end point is reached. Usually more time is required for the mercury to become stationary in the case of plant tissues than it is with soils. The work may be greatly facilitated by having one or two tubes of material in ice water while the freezing point of another sample is being ascertained.

After one has acquired skill in making the determinations, concordant results may be obtained with very little difficulty. As a general rule, different samples of the same material do not vary in their freezing-point lowerings more than .01° C. and in many cases they are identical.

Before taking up the studies of relations between the water content of soils, the composition and concentration of the soil solution, and the concentration of the cell sap of roots and tops of plants and plant growth, it is logical to present certain data we have obtained which we consider to have justified our methods of procedure throughout our researches herein reported.

Previous to our work above referred to two methods were widely employed to determine the concentration of cell sap of plants, namely, the plasmolytic method (3, 7, 13, 16, 25, 27) and the freezing point of extracted liquid method (2, 5, 6, 8, 9, 10, 14, 18, 22, 26). The conductivity method has been used to some extent but since it has but little bearing upon the work presented we have omitted any discussion of it. Although a brief review of the literature bearing directly on this problem—that we have been able to obtain—is presented later on, it is well to consider at this time the work of Müller-Thurgau (22), and Cavara (5).

Müller-Thurgau, before the advent of the Beckmann thermometer, made rather extensive studies of the freezing-point lowerings of the fruits of apple, pear and grape, as well as potato tubers and leaves of the bean. He determined the depression of the extract of some, of the triturated tissue of others, and the undisturbed tissue in certain cases, by simply inserting the bulb of the thermometer into it.

Cavara made determinations of the freezing-point lowerings of the expressed sap of many species of plants. In some cases, where it was difficult to obtain the cell sap the bulb of the Beckmann thermometer was inserted into the tissue and in others the freezing-point lowerings of the macerated tissue were determined.

The Effect of Different Methods of Treatment of Plant Tissue upon the Freezing-Point Lowerings

In order to determine whether or not the results obtained from the direct freezing method are similar, or strikingly different from those derived after having subjected the mass of plant tissue to repeated freezing, to low temperatures, to low temperatures and trituration, and to low temperatures and extraction under great pressure, respectively, the experiments reported in the immediately following paragraphs were performed.

TABLE I

THE EFFECT OF REPEATED FREEZING UPON THE CONCENTRATION

Times frozen	Corn Roots		Corn Tops			
			A		B	
	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres
1	.430	5.182	.640	7.710	.625	7.520
2	.425	5.122	.600	7.229	.624	7.510
3	.435	5.242	.635	7.650	.625	7.520
4	.430	5.182	.633	7.630	....	.....

Times frozen	Pea Roots				Pea Tops			
	A		B		A		B	
	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres
1	.865	10.420	.885	10.660	1.250	15.04	1.251	15.05
2	.835	10.055	.890	10.720	1.275	13.34	1.252	15.06
3	.850	10.240	.865	10.420	1.250	15.04	1.270	15.28
4	.842	10.144	.870	10.480	1.268	15.26	1.264	15.20

	Clover Roots				Clover Tops			
1	.425	5.122	.445	5.362	.825	9.935	.795	9.574
2	.420	5.062	.435	5.242	.830	9.995	.780	9.394
3	.425	5.122	.440	5.302	.815	9.815	.805	9.695
4	.430	5.182	.425	5.122	.820	9.875	.785	9.454

Repeated Freezings. Roots and leaves of corn, Canada field pea, rye, and clover, respectively, were prepared and placed in freezing tubes in the usual manner, supercooled one degree, and the freezing point repeatedly determined without removing the Beckmann thermometer from the mass during the procedure. By so doing, changes in the water content of the material in the tube were prevented. The results obtained are to be found in Table I.

The variations in the above results lie practically within the experimental error and, therefore, we are justified in concluding that repeated supercooling and freezing did not alter the lowering of the freezing points of the different substances studied.

Repeated Exposure to Low Temperatures. We next conducted experiments to determine if the exposure of different plants to low temperatures appreciably affect their freezing points as determined by the direct method. The plants, or parts of plants, utilized in these studies were placed in tubes which were securely corked, and inserted into the ice and salt mixture, the temperature of which was 10 degrees below zero. The tubes were removed after the contents had solidified (which usually required about one hour), the mass thawed and the freezing point determined in the usual manner. It was necessary to remove the thermometer each time the tubes were placed into the cooling mixture in order to prevent it from being broken by the solidification of the mass. The data obtained are given in Table II.

TABLE II

THE EFFECT OF REPEATEDLY SUBJECTING PLANT TISSUES TO LOW TEMPERATURES UPON THE FREEZING-POINT LOWERINGS

Times frozen	Corn Leaves				Pea Leaves			
	A		B		A		B	
	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres
0	.532	6.406	.585	7.048	.580	6.988	.585	7.048
1	.538	6.486	.587	7.078	.585	7.048	.590	7.108
2	.562	6.767	.578	6.978	.590	7.108	.600	7.229
3	.565	6.847	.590	7.108	.620	7.469	....	.....
4	.575	6.928	....	.....	....	.....	....	.....
	Corn Roots				Pea Roots			
1	.465	5.603	.470	5.664	.248	2.985	.255	3.074
2	.470	5.664	.485	5.844	.255	3.074	.265	3.194
3	.475	5.724	.490	5.904	.265	3.194	.280	3.375
4	.485	5.844	.496	5.976	.270	3.255	.285	3.435

As in the previous series the differences in the results obtained seem to be the result of errors that are introduced. One error, that is impossible to prevent, is the removal of an appreciable amount of liquid on the surface of the thermometer each time it is taken from the freezing tube. Moreover, it is possible that enzymotic activities bring about some changes in the cell contents.

Low Temperature and Trituration. Comparisons of the freezing points of tissue determined directly and subjected to low temperatures

and to trituration were made. Usually aluminum test tubes were employed as the freezing tubes. Unless the material was put through a food grinder while solidified, it was thoroughly macerated by means of a sharpened steel rod while in the aluminum tubes. Great care was taken to prevent changes in the water content of the material, the containers being tightly stoppered at all times, except when it was necessary to insert the thermometer, or the steel rod. The data resulting from these experiments are given in Table III.

TABLE III

THE EFFECT OF LOW TEMPERATURES AND TRITURATION UPON THE FREEZING-POINT LOWERINGS OF DIFFERENT MATERIALS

Material	Treatment	Freezing-point depression	Osmotic pressure atmospheres
Potato tubers.....	Direct freezing	0.550	6.628
		0.555	6.688
	Frozen solid and macerated to a cream.	0.572	6.887
		0.552	6.648
	Frozen solid and ground	0.565	6.807
		0.570	6.867
Potato tubers.....	Direct freezing	0.518	6.245
		0.565	6.807
	Frozen solid and macerated to a cream.	0.570	6.867
		0.552	6.648
	Frozen solid and ground	0.577	6.938
		0.552	6.648
Clover Leaves.....	Direct freezing	1.018	12.26
		1.045	12.58
	Frozen solid and thoroughly triturated.	1.045	12.58
		1.070	12.88
	Frozen solid and ground	1.117	13.44
		1.102	13.27
Canada Field Pea Leaves	Direct freezing	0.552	6.648
		0.550	6.628
	Frozen solid and thoroughly triturated.	0.565	6.807
		0.558	6.728
Canada Field Pea Roots.	Direct freezing	0.351	4.229
		0.355	4.279
	Frozen solid and triturated	0.372	4.479
		0.360	4.339

An examination of the above data reveals that there is nothing to be gained by subjecting the material to low temperatures and to trituration, inasmuch as the results obtained practically agree. It is noticeable that the results obtained by grinding the frozen material are slightly higher than those obtained by trituration directly in the freezing tube. This is doubtless due to unavoidable loss of moisture during the process of grinding. It is well to mention that the plants used in the above studies, with the exception of clover, were quite succulent.

Low Temperature, Trituration and Extraction. Results reported by Dixon (10) show conclusively that the freezing point of pressed vegetable juices varies with the treatment of the tissue before being pressed, as well as with the degree of extraction. (We have verified the latter point.) Exposure of the tissue to chloroform, to toluene, to heat, to intense cold, and to desiccation and remoistening, respec-

TABLE IV

COMPARISONS OF THE FREEZING-POINT LOWERINGS OF PLANT TISSUE DETERMINED DIRECTLY AND OF THE EXTRACTED LIQUID

Crop	Direct Freezing		Expressed Liquid	
	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres
TOPS				
Oats	1.620	19.480	1.690	20.320
	1.640	19.720	1.690	20.320
Rye	0.890	10.720	0.825	9.935
	0.900	10.840	0.830	9.995
	0.690	8.312	0.740	8.913
	0.700	8.432	0.720	8.672
	0.853	10.280	0.835	10.050
	0.830	9.995	0.825	9.935
ROOTS				
Oats	1.110	13.360	0.895	10.780
	1.120	13.480	0.895	10.780
	1.110	13.360	0.890	10.720
	1.090	13.120	0.880	10.600
	0.380	4.580	0.320	3.857
	0.390	4.700	0.330	3.978
	0.180	2.171	0.190	2.291
	0.180	2.170	0.200	2.412
Barley	0.975	11.740	0.955	11.500
	0.970	11.680	0.950	11.440
Corn	0.412	4.965	0.355	4.279
	0.440	5.302	0.350	4.219

tively, before being pressed, materially increases the lowering of the freezing point of the extracted sap over that obtained from untreated tissue. The author suggests that the liquid pressed from untreated vegetable tissues is not an average sample of the sap contained in the cells of those tissues, but the exposure to intense cold renders the cells permeable and the liquid is not altered in concentration by extraction. It was considered advisable to compare the freezing-point lowerings

of liquid that had been extracted from tissue subjected to intense cold with that of the freezing-point lowerings of the tissue. The mass to be subjected to low temperature was placed in pint mason jars, which were carefully sealed and buried in a freezing mixture and left for 3 hours. Following this exposure the material was at once placed in the press and subjected to a pressure of 300 kg. per square centimeter until all liquid was removed under these conditions. The freezing point of the extract was determined immediately. Both roots and leaves of different plants of high and of low water content and depression have been used in making comparisons. The data obtained are given in Table IV.

As indicated by the data presented in Table IV the freezing-point lowerings of the leaves of the plants studied are practically the same as those of the extracted liquid. We found that precautions must be taken to reduce to the minimum the change in water content of the tissue while being prepared for and during the process of extraction.

The results obtained from the studies of the roots are not so consistent, in some cases the extracted liquid affording less depression than the tissue frozen directly, and in others practically the same, but the differences are greatest in cases where the depressions are great. At present we are unable to account for such variations unless they are due to adsorption in some cases by the solid mass.

The Rate of Water Translocation in Soils as Measured by the Freezing-Point Lowerings of Roots and Tops of Plants

Early in our work it was found difficult to obtain concordant results with samples of tops of plants taken from the soil at various intervals during the day. Dixon (8) noted that illumination increased the osmotic pressure in the cells of leaves, and conversely, he found that the pressure gradually fell when the leaves were cut off from the light. The difference thus produced amounted to more than 14 atmospheres in some instances. He attributes such differences chiefly to the formation of soluble carbohydrates in the light, thus increasing the concentration of the cell sap. Later on Chandler (6) established that when plants are shaded 24 hours the sap density materially decreases. It is well to call attention to the fact that neither of these investigators presents data to show whether or not the change in concentration is due mainly to the formation of carbohydrates, or to some other condition, such as the rate of water supply by the soil and consequently a change in the water content of the tissue.

Inasmuch as such changes have a very important bearing upon the methods of procedure in our later work, and in view of the fact that they throw light upon the moisture relations of soils and plants, numerous determinations of the concentration of the cell contents of the roots and tops of certain plants were made. In all cases reported, the samples were

placed in the freezing tubes and the freezing point determined immediately. Inasmuch as changes in the water content of plant tissues may alter the concentration of the cell contents markedly; the total loss from the leaves upon drying in an oven heated to 105 °C. was determined. The writers fully appreciate that the total loss in weight so determined may not truly represent the amount of active water present. It may be cited, for example, that Müller-Thurgau (22), many years ago showed that much of the total plant water as determined by loss of weight upon drying may not be frozen and the amount varies with the intensity of the cold to which plants are exposed.

The first series of experiments conducted were under field conditions and no precaution was taken to alter the light intensities. The results of these studies appear in Table V.

TABLE V

VARIATIONS IN THE CONCENTRATION OF THE CELL SAP OF THE LEAVES OF PLANTS AT DIFFERENT INTERVALS DURING THE DAY

Hour of Sampling	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres
CORN LEAVES			
6.00 a. m.	83.56	.544	6.566
12.00 noon	80.47	.778	9.370
6.00 p. m.	82.56	.739	8.903
12.00 midnight	83.23	.607	7.309
CANADA FIELD PEA LEAVES			
6.00 a. m.	90.72	.585	7.048
12.00 noon	87.75	.760	9.154
6.00 p. m.	87.78	.630	7.590
12.00 midnight	88.90	.570	6.867

The results of these studies show very strikingly that the freezing point of the tissues was greatest about noon, appreciably lower at 6.00 p. m., and still lower at midnight, and usually lowest early in the morning, or about sunrise. Moreover, the loss upon drying was found to be greatest when the concentration of the cell sap was lowest. There seems to be, then, a close correlation between our results and the rate of water movement from the soil to the plant at different hours of the day as determined by Briggs and Shantz (4), as well as the results of Lloyd's (21) studies of the leaf water of cotton.

It is assumed that photosynthesis also played some rôle in the change in the concentration of the cell contents. Yuncker (28) recently reported that the rate of photosynthesis of the corn plant grown in the greenhouse is greatest at noon and gradually decreases from this time to sunset.

In order to throw additional light upon this question, comparative studies were made with plants that were shaded by means of an inverted

box placed over the plants when the first samples were taken in the morning, and those exposed. These experiments were conducted under field conditions. The data obtained appear in Table VI.

TABLE VI

VARIATIONS IN THE CONCENTRATION OF THE CELL SAP OF THE LEAVES OF PLANTS AT DIFFERENT INTERVALS (FIELD STUDIES)

Time of Sampling and Weather Conditions	Air Temperature ° C.	Freezing point depression	Osmotic pressure atmospheres	Water content per cent
OATS, UNSHADED				
5.30 a. m. Partly cloudy	12.25	0.681	8.201	86.28
9.15 a. m. Completely cloudy ..	17.50	0.755	9.093	86.18
1.00 p. m. Hazy sunshine	20.25	0.889	10.710	83.78
5.00 p. m. Cloudy	18.25	0.878	10.580	84.77
5.30 a. m. Hazy sunshine	12.00	0.650	7.830	87.46
9.15 a. m. Partly cloudy	14.25	0.758	9.134	86.57
1.15 p. m. Cloudy	16.00	0.757	9.124	86.00
5.00 p. m. Misting rain	13.00	0.758	9.134	85.68
OATS, SHADED				
5.30 a. m. Partly cloudy	12.25	0.681	8.201	86.28
9.15 a. m. Completely cloudy ..	.....	.....	.....	.....
1.00 p. m. Hazy sunshine	20.00	0.638	7.690	87.37
5.00 p. m. Cloudy	17.50	0.623	7.509	88.19
5.30 a. m. Hazy sunshine	12.00	0.650	7.830	87.46
9.15 a. m. Partly cloudy	.....	.....	.....	.....
1.15 p. m. Cloudy	15.00	0.604	7.279	87.95
5.00 p. m. Misting rain	13.00	0.597	7.208	88.42
RYE, UNSHADED				
5.30 a. m. Fair	13.00	0.790	9.514	78.99
9.30 a. m. Fair	24.00	0.942	11.350	76.61
1.15 p. m. Fair	25.00	1.014	12.200	76.23
5.00 p. m. Hazy	22.00	1.032	12.420	76.62
5.30 a. m. Cloudy	18.50	0.768	9.244	79.87
9.15 a. m. Hazy sunshine	22.75	0.955	11.500	75.74
1.00 p. m. Hazy sunshine	25.00	1.012	12.190	75.79
5.00 p. m. Hazy sunshine	22.50	0.788	9.504	76.55
RYE, SHADED				
5.30 a. m. Fair	13.00	0.790	9.514	78.99
9.30 a. m. Fair	.....	.....	.....	.....
1.15 p. m. Fair	31.00	0.711	8.562	79.25
5.00 p. m. Hazy	21.00	0.706	8.502	80.09
5.30 a. m. Cloudy	18.50	0.768	9.244	79.87
9.15 a. m. Hazy sunshine	.....	.....	.....	.....
1.00 p. m. Hazy sunshine	26.50	0.755	9.093	80.68
5.00 p. m. Hazy sunshine	22.50	0.784	9.444	80.97

The results obtained show that the moisture content of the leaves of the plants in the shade remains quite constant throughout the day, but that

the concentration of the cell sap showed a tendency to become more dilute from 5.30 a. m. to 5.00 p. m. Moreover, the decrease in the water content of the exposed plants apparently does not account *in toto* for the increase in concentration of the cell sap or, in other words, the products resulting from photosynthesis probably play their part.

Although this question is quite incidental to the problem we had under investigation, a series of experiments with potted plants was run in order to obtain additional information concerning it.

In these studies, the plants were kept in a saturated atmosphere by inverting a large bell jar over them. The results presented in Table VI show that the concentration of the cell sap of the leaves was lowest at sunrise and greatest at 1.00 p. m., and, furthermore, indicate that these differences are not due wholly to the water content of the tissue. Moreover, the concentration of the roots varied, but less strikingly than that of the tops, and in a reverse order with respect to time of sampling. Similar results were obtained with peas.

TABLE VII

FREEZING-POINT LOWERINGS OF ROOTS AND LEAVES OF THE CORN PLANT AT DIFFERENT INTERVALS DURING THE DAY

Time	Tops			Roots	
	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres
Sunrise	.503	6.061	93.4	.375	4.519
9.00 a. m.	.570	6.867	91.8	.388	4.676
1.00 p. m.	.625	7.529	91.5	.336	6.050
5.00 p. m.	.570	6.867	92.7	.450	5.423
10.00 p. m.	.558	6.724	92.8	.359	4.327

Experimental

The freezing-point lowerings of the roots and tops of plants as a measurement of the composition and concentration of the soil solution, as well as the different water contents and rate of water movement in soils, have been studied. Several of the experiments herein reported were conducted in a greenhouse but many were carried on under field conditions.

The Freezing-Point Lowerings of Plants as Indicators of the Concentration of the Solution in Which They Grow

Certain investigators have established that there is some relationship between the concentration of the media in which plants grow and their cell sap. Weiler (27) early determined the concentration of the cell sap of *Helianthus annus, Vicia faba* and *Phaseolus multiflorus* by the plasmolytic method and reported that the roots and tops were the same in concentration, and that the strength of sugar required to cause plasmoly-

sis lies between 6 and 7 per cent. He also showed that stronger solutions were required to bring about plasmolysis after the plants had been in contact with sugar solutions of higher concentrations several hours.

Copeland (3) employing solution cultures presented data which show that the cell sap of the roots and leaves of *Phaseolus multiflorus, Phaseolus vulgaris, Pisum sativum, Sinapsis alba, Fagopyrum* and *Zea Mays* is altered by the composition and the concentration of the solutions in which they grew. He also found that the cell sap of the roots in some instances is slightly less concentrated than that of the aerial portions of the plants.

Drabble and Drábble (11) report that the osmotic pressure of the sap in turgid cells in the plants studied (total number being 48), ranges from 3386.6 to 1495.13 mm. of mercury. In addition they found the osmotic strength of the sap of plants growing in fresh water marshes to be lower than that of those growing in salt water marshes. Moreover, the concentration increases with the degree of physiological drouth, and in any area it varies with the physiological scarcity of water, but plants growing under the same conditions generally have the same concentration of cell sap. Moreover, "the depression of the freezing point of the sap within the range of strengths found will be small, but may be effective in the case of plants with the highest osmotic strength of sap."

Hill (19) in studies with salt marsh plants found that the root hairs vary their concentration with the strength of the soil water and the concentration of the different root hairs, varies on the same plant. Moreover, different individuals of the same species likewise vary in this respect.

Bouyoucos (1) noted a close relation between the density of the cell sap of wheat and bean seedlings, and the outside solution in which they were grown.

The senior author made many determinations of the concentration of the cell sap of *Tradescantia Zebrina* that had grown 30 to 60 days in distilled water, full nutrient solution, and single and mixed salt solutions, respectively. Unpublished data show very strikingly that the density of the solutions in which the cuttings grow appreciably influences the osmotic pressure of the cell sap and indicate that the composition also influences it.

In view of the fact that the direct freezing point method has never been used, to our knowledge, to determine whether or not the concentration of the cell sap of roots and leaves of plants is indicative of the density of the solutions in which they grow, several experiments were conducted in the greenhouse with Canada field pea and wheat seedlings.

The three-salt solution of Shive (24) was employed. The concentration ranged from one-tenth to four times that suggested by this investigator. The experiments were continued 17 and 24 days in the case of

peas, and 14 and 30 days, respectively, with wheat seedlings. Several hours previous to the freezing-point determinations, the tops were rinsed in distilled water and allowed to dry, the roots remaining in contact with the solution. The roots of the plants were likewise rinsed and carefully dried between sheets of filter paper before freezing. In all cases the loss upon drying at 105° C. was recorded. The results obtained from these studies are presented in Table VIII.

TABLE VIII

THE FREEZING-POINT LOWERINGS OF ROOTS AND LEAVES OF PLANTS, AS A MEASUREMENT OF THE DENSITY OF THE CULTURAL SOLUTIONS

	Tops			Roots		
Strength of solution	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent
CANADA PEA SEEDLINGS, 17 DAYS OLD						
4 x Nutrient	.673	8.101	91.97	.424	5.102	95.23
1/10 Nutrient	.543	6.536	93.38	.371	4.469	93.64
Nutrient	.549	6.616	93.42	.369	4.449	95.47
2 x Nutrient	.608	7.319	93.47	.405	4.881	95.67
CANADA PEA SEEDLINGS, 24 DAYS OLD						
1/10 Nutrient	.613	7.379	92.24	.328	3.947	93.11
Nutrient	.650	7.830	92.53	.353	4.249	95.24
2 x Nutrient	.712	8.572	94.38	.404	4.861	95.34
4 x Nutrient	.790	9.514	92.09	.424	5.102	95.35
WHEAT SEEDLINGS, 14 DAYS OLD						
1/10 Nutrient	.641	7.720	90.53	.181	2.181	94.48
Nutrient	.780	9.394	93.05	.360	4.339	94.82
2 x Nutrient	.861	10.370	93.07	.391	4.710	94.53
4 x Nutrient	.887	10.680	92.37	.374	4.499	94.89
WHEAT SEEDLINGS, 30 DAYS OLD						
1/10 Nutrient	.748	9.003	90.71	.402	4.841	92.44
Nutrient	.835	10.050	91.94	.592	7.128	92.63
4 x Nutrient	.995	11.980	91.37	....	.....	.....

The data show conclusively that the freezing point of the roots and tops of Canada field pea and wheat seedlings is indicative of but not directly proportional to the density of the nutrient solution in which they are grown. Although the conditions for growth were identical, the concentration of the cell sap of the two plants, as measured by the direct freezing point method, differed appreciably. The tops of the wheat seedlings gave greater depressions than those of the pea seedlings at the close of each period. Moreover, the roots of the former showed greater difference in concentration at the close of the second period than the roots of the latter. It is well to note that in all cases the roots were lower in concentration than the tops, undoubtedly due in part at least to a higher water content.

The early work of Copeland (3) with solution cultures point to similar relations between concentration of the sap of the roots and tops of seedlings. On the other hand, Stange (25) did not report difference in the osmotic pressure of the stems and of the roots of *phanerogamia*. Dixon (10) by means of the thermo-electrical method found sap expressed from certain plants to range in pressure from 11.6 to 16.9 atmospheres, but that taken from roots of the same plants gave pressures of 4.3 to 5.9 atmospheres. At about the same time, Nathonson (23) discussed this relationship. More recently, Hanning (16) employing the plasmolytic method determined the concentration of the cell sap of the roots and leaves of a large number of plants, the total number being 62. With few exceptions the sap of the leaves showed greater concentrations than that of the roots.

In connection with the difference in depression exhibited by the cell sap of the aerial portion of the plants studied and root juice, the question suggests itself, whether there is an actual difference in the amount of soluble material present, or is it possible that there is a difference in the amount of water that freezes?

TABLE IX

A COMPARISON OF THE LOSSES ON DRYING THE ROOTS AND TOPS OF PLANTS AT 105° C. AND OF THE AMOUNTS OF WATER IN THE MATERIAL THAT FREEZES

Plant: Barley	Tops		Roots	
	Percentage loss at 105° C.	Percentage water that froze	Percentage loss at 105° C.	Percentage water that froze
Morning	80.32	55.06	88.33	61.51
	80.43	54.63	86.84	62.91
Noon	80.47	42.22	82.09	57.08
	81.63	44.46	85.89	60.78

To throw some light on this point the amount of water that freezes in the plant tissue was measured in the following manner. A weighed sample of the material, about 10 gm., was thoroughly triturated in a dilatometer[1] by means of a sharpened steel plunger, such as was used for crushing similar material in the freezing point tubes. The bowl of the dilatometer was then filled with ligroin. It required considerable time to expel air from the interstices of the tissue but with thorough trituration and due care replacement ceased. The instrument was placed in a bath about —3° to —4° C. and allowed to remain undisturbed until the contents reached the same temperature as the bath, as shown by no further shrinkage of the ligroin in the stem of the dilatometer. By agitating the instrument solidification was now induced and the expansion measured.

[1] Dr. Geo. J. Bouyoucos is directly responsible for the development of dilatometer method. His work along these lines is now in the process of publication.

The percentage of water that freezes and the percentage of loss on drying the material at 105° C. is shown in Table IX.

The data presented indicate a striking difference in the per cent of water freezing in the roots and tops of barley plants, and also a marked difference in the per cent of water freezing in the tops in the morning and at noon. The plants were kept under such conditions that transpiration was retarded while photosynthesis could proceed. It seems probable that the physiological activities of the cells play an important rôle in the amount of free water being decreased.

An additional series was conducted with Canada pea seedlings. In these experiments soil extracts were used which were obtained from both virgin and cropped sandy loam and silt loam soils in the usual manner, that is by shaking with five parts of distilled water, allowing to settle and then filtering through Chamberland-Pasteur filters. Three-gallon jars were used as containers, the seedlings being inserted through holes in heavily paraffined paper, which covered the jars, into the solution. At the close of 14 and 25-day periods the freezing-point lowerings of the roots and tops of the plants were determined. The data obtained are presented in Table X.

The data presented show no apparent relation between the type of soil from which the extract was obtained and the freezing-point depression of the roots and tops of the plants grown therein. In the case of the roots there seems a tendency for those growing in the extract from the cropped soil to have a slightly higher freezing-point depression than those growing in the extract from the virgin soil of the same type.

Since the freezing-point depressions of the various extracts are practically the same, it seems that any variation in the freezing-point depression of the plants must be due to the composition of the extracts, or to some other uncontrolled factor.

The Concentration of the Soil Solution as Measured by the Freezing-Point Lowerings of the Roots and Tops of Plants

Since the results derived from solution cultures show clearly that the density of the same is indicated by the freezing-point lowerings of roots and tops of certain plants grown therein, the question at once arises as to whether or not similar conditions obtain with respect to the soil and plants. In order to obtain further evidence a series of soil cultures was prepared.

Sandy loam was placed in 1-gallon pots, the moisture maintained uniform and constant, throughout the experiment, while the concentration of the soil solution was varied by the addition of soluble salts. The experiments were run in triplicate, but the results obtained are reported in duplicate. Previous to freezing, the roots and tops were rinsed with distilled water and carefully dried between layers of filter paper. Samples of each were brought to constant weight in an oven heated to 70° C. and

TABLE X

THE FREEZING-POINT LOWERINGS OF PLANTS GROWN IN SOIL EXTRACTS

ROOTS

Soil	Period of Growth 14 Days				Period of Growth 25 Days		
	Freezing-point depression of the extract	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent
Sand virgin	.009	.310 .312	3.737 3.757	95.08	Not	run	
Sand cropped	.015	.375 .358	4.519 4.319	94.18	.300 .305	3.616 3.676	93.66
Sandy loam virgin..	.009	.430 .395	5.182 4.760	94.20	.330 .335	3.978 4.038	93.34
Sandy loam cropped	.011	.430 .419	5.182 5.052	94.58	.365 .365	4.399 4.399	94.49
Silt loam virgin....	.011	.300 .305	3.616 3.676	94.56	.320 .310	3.857 3.737	95.88
Silt loam cropped...	.010	.335 .325	4.038 3.917	94.04	.390 .395	4.700 4.760	91.86

TOPS

Sand virgin	.009	.575 .572	6.927 6.897	89.26	Not	run	
Sand cropped	.015	.555 .550	6.788 6.628	90.14	.605 .615	7.289 7.409	87.16
Sandy loam virgin..	.009	.625 .620	7.529 7.469	88.84	.715 .720	8.612 8.672	87.98
Sandy loam cropped	.011	.600 .617	7.229 7.439	89.64	.590 .605	7.108 7.289	93.58 ?
Silt loam virgin....	.011	.535 .542	6.446 6.526	89.30	.710 .700	8.552 8.432	86.20
Silt loam cropped...	.010	.560 .550	6.747 6.628	88.78	.725 .738	8.732 8.893	86.30

the total loss in weight recorded. For 24 hours previous to freezing the plants were not exposed to light.

The concentration of the tops of the plants that grew in the soil to which the full treatment was afforded, was slightly greater than that of those grown in the soil which received only one-half the ration of soluble salts, as well as those in the untreated soil. On the other hand, by the freezing point of the tops the concentration of the soil solution found to exist in the two latter soils was not indicated. In other words, all other conditions being maintained as nearly the same as possible, only rather large variations with respect to the soil solution are indicated by the freezing-point lowerings of the tops of plants. The results also show that the roots of the plants grown under the above conditions are better indicators of the concentration of the soil solution than the tops, the freezing-point lowerings decreasing with, but not directly proportional to the decrease in density of the soil solution with which they come in contact.

TABLE XI

THE FREEZING-POINT LOWERINGS OF ROOTS AND TOPS OF PLANTS AS A MEASUREMENT OF THE CONCENTRATION OF THE SOIL SOLUTION

Treatment per Acre	Tops			Soil	Roots		
	Freezing-point-depression	Osmotic pressure atmospheres	Water content per cent	Moisture per cent	Freezing-point-depression	Osmotic pressure atmospheres	Water content per cent
$MgSO_4$ 300 lbs....	.537	6.466	89.32	10.08	.434	5.232	90.53
$NaNO_3$ 300 lbs. ...	.535	6.446	88.83	8.82	.483	5.814	89.62
K_2HPO_4 500 lbs...	.562	6.767	89.42	10.25	.445	5.362	89.70
Average	.545	6.566	89.19	9.72	.454	5.473	89.95
½ Treatment	.504	6.075	89.78	10.85	.428	5.162	87.82
	.509	6.135	90.00	9.28	.421	5.072	89.83
	.533	6.426	89.37	10.11	.431	5.192	89.24
Average	.515	6.205	89.72	10.08	.427	5.152	88.96
Check	.540	6.506	87.83	11.25	.365	4.399	88.64
	.519	6.245	88.89	10.53	.410	4.941	88.67
	.519	6.245	88.79	12.26	.357	4.299	89.45
Average	.526	6.336	88.50	11.35	.377	4.539	88.92

An additional series of experiments was conducted. Jars of 3-gallon capacity were filled with silt loam soil, the same amount of water was added to each, and maintained constant by means of an inverted funnel that extended to within about 6 inches of the bottom of the jar and glass tubes that penetrated through the wax seal. The concentration of the soil solution was varied markedly by additions of different amounts of a strong solution of calcium nitrate, magnesium sulfate, and potassium phosphate. In this series the roots of the plants were freed of as much soil as possible by vigorous shaking before they were frozen. The

error so introduced, due to the presence of the soil particles on the roots, is probably very slight, as many determinations seem to indicate.

The concentration of the tops of the plants proved to be less valuable indicators of the concentration of the soil solution than the roots. Consequently, there was less difference between the concentration of the roots and tops of plants grown in the soils possessing high concentration; indeed, the roots of the plants removed from cultures 4 and 5 afforded greater depressions than the tops. The data obtained appear in the table below.

TABLE XII

THE FREEZING-POINT LOWERINGS OF ROOTS AND TOPS OF PLANTS AS INDICATORS OF THE CONCENTRATION OF THE SOIL SOLUTION

Culture No.	Soil			Corn Tops			Corn Roots	
	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres
1	.101	1.216	16.18	.511	6.155	89.89	.381	4.590
2	.165	1.990	16.56	.585	7.048	89.50	.455	5.483
3	.281	3.385	16.99	.577	6.957	90.00	.549	6.618
4	.412	4.961	17.20	.601	7.239	89.46	.624	7.509
5	.600	7.229	17.22	.605	7.289	90.16	.680	8.191

In order to throw additional light upon this question, nutrient salts were added to pots of sandy soil in which were growing rye plants about one foot in height, in sufficient amounts to increase markedly the concentration of the soil solution. The jars were sealed and remained outside the laboratory, and the soil permitted to become dry, without further addition of water. In the meantime the concentration of the soil solution, the water content of the soil as well as the freezing-point lowerings of the roots and tops of the plants were determined. The data presented in Table XIII show very strikingly that the concentration of the soil solution is rather closely correlated with the freezing-point lowerings of the roots of the plants in contact with it, but the tops of the plants are less sensitive to changes in the concentration of the soil solution.

Moisture Relations of Soils and Plants

According to Drabble and Lake (10) a greater concentration of cell sap occurs in those plants which are most strongly subjected to factors tending to promote loss of water by transpiration. Livingston (20) concludes, from certain of his studies, that the concentration of the cell sap of desert plants is not appreciably greater than that of ordinary land plants, while Fitting (13) maintains, as a result of his investigations, that the concentration of the sap of epidermal leaves taken from desert plants is very great, ranging from 30 to 100 atmospheres, and that the root sap of the same must likewise be very highly concentrated. Hibbard and

Harrington (18) report, as a result of a single series of experiments, that as the water content of a soil decreases the depression of the freezing point of both tops and roots of plants increases.

TABLE XIII

THE CONCENTRATION OF THE SOIL SOLUTION AS INDICATED BY THE FREEZING-POINT LOWERINGS OF THE ROOTS AND TOPS OF PLANTS

Soil			Tops			Roots	
Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic atmospheres pressure	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres
.285	3.435		1.240	14.92		0.555	6.688
.285	3.435	9.60	1.240	14.92		0.555	6.688
.500	6.025		1.420	17.08		1.250	15.04
.495	5.904	4.10	1.440	17.32	78.66	1.240	14.92
.890	10.72		1.420	17.08		0.870	10.48
.900	10.84	3.25	1.405	16.90	80.70	0.850	10.24
*	.....		1.660	19.96		1.450	17.44
*		2.18	1.670	20.08	79.92	1.470	17.68
*	.....		1.400	16.84		2.060	24.75
*		2.06	1.420	17.08	74.53	2.050	24.63

* Did not freeze.

Now the question arises—Is the increase in concentration brought about, mainly, the result of slow movement of water from the soil to the

TABLE XIV

THE FREEZING-POINT LOWERINGS OF ROOTS AND LEAVES OF THE CORN PLANT AS INDICATORS OF THE WATER CONTENT OF SOILS

Soil Condition	Soil			Leaves			Roots	
	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Osmotic pressure atmospheres	Water content per cent
High water content	.0170	0.201	22.3	.522	6.280	89.4	3.43	86.5
	.0210	0.251	....	.504	6.130	89.4	4.34	....
	.0150	0.181	18.8	.531	6.390	88.7	3.67	89.9
Average	.0177	0.211	20.6	.521	6.267	89.2	3.82	88.2
Low water content	.5140	6.180	8.0	.505	6.080	88.9	6.56	87.8
	.5210	6.270	7.7	.490	5.900	88.4	5.81	87.6
	.4570	5.510	10.1	.503	6.050	87.8	5.77	88.2
Average	.4970	5.980	8.6	.499	6.010	88.4	6.03	87.8

plant, or is it the result of greater density of the soil solution, or of both? It is rather difficult to settle this point, inasmuch as we have shown that the concentration of the soil solution may be altered quite strikingly by

changes in the water content. Many experiments have been conducted to throw light upon this question. The results of certain of these are presented in Table XIV.

Silt loam was placed in 3-gallon jars, the water content was maintained slightly above the critical content in three, and near the so-called optimum content in three others. The corn plant was employed as the indicator, the duration of the experiment being 35 days. The roots of the plants were rinsed and dried between filter paper before the freezing points were determined. The losses in weight of tops and roots upon drying in an oven heated to 105° C. were also ascertained.

The results presented in Table XIV show clearly that the water content of the soil and therefore the concentration of the soil solution is closely correlated with that of the cell sap of the roots of the plants growing therein.

The next series of experiments conducted under greenhouse conditions, although of short duration, affords additional information concerning this question. Sandy loam was placed in pots, the water content being maintained constant at 8, 12, and 16 per cent, respectively.

TABLE XV

THE WATER CONTENT OF SOILS AS INDICATED BY THE FREEZING-POINT LOWERINGS OF ROOTS AND LEAVES OF THE CORN PLANT
(Duration of experiment 14 days)

Water content of soil	Soil		Tops		Roots	
	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres
	.555	6.506	.530	6.386	.560	6.747
8%	.540	6.688	.535	6.446	.567	6.837
	.220	2.652	.506	6.085	.435	5.242
12%	.235	2.832	.520	6.266	.445	5.362
	.135	1.518	.520	6.266	.470	5.664
16%	.150	1.809	.515	6.205	.480	5.784

The above results show less striking differences with respect to concentration of the roots of the plants grown in the soils of different water contents. It is a notable fact also that the concentration of the soil solution did not vary so markedly as in the previous series. It should also be noted that the soil was removed, as far as possible, by shaking the roots. The freezing-point lowerings of the leaves are practically the same throughout, those grown in the soil of lowest water content being very slightly higher than the others.

On November 1, rye plants about one foot in height, growing in groups of 50 or more, were transferred with the immediately surrounding soil to glazed jars. The soil was kept moist 10 days by additions of water. At the end of this period the jars were sealed and the soil permitted to

become dry by loss of water through the leaves of the plants. The freezing point of the roots and tops, as well as the water content and the freezing-point lowerings of the soil were determined at successive stages until the plants were completely wilted. In this manner the moisture relations of the soil and plants were determined.

TABLE XVI

CHANGES IN SOIL MOISTURE AS INDICATED BY THE ALTERED FREEZING-POINT LOWERINGS OF THE ROOTS AND TOPS OF PLANTS

Soil			Tops			Roots	
Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres
.06	0.724		1.240	14.92		0.312	3.757
.07	0.844	....	1.240	14.92	.....	0.317	3.827
.22	2.652		1.170	14.08		0.600	7.229
.22	2.652	4.44	1.150	13.84	.....	0.585	7.048
.22	2.652	4.12	1.190	14.32		0.590	7.108
.23	2.772		1.230	14.80	.....	0.560	6.747
*			1.310	15.76		1.275	15.340
*	.....	2.01	1.360	16.36	79.57	1.280	15.400
*			1.770	21.28		1.300	15.640
*	.....	2.01	1.780	21.40	75.98	1.300	15.640
*			1.670	20.08		1.330	16.000
*	.....	2.21	1.660	19.96	75.88	1.300	15.640

* Did not freeze.

The data in Table XVI indicate that there is no relation between the water content of the soil and the freezing-point lowerings of the leaves of the plants growing in it until the critical water content of the soil is reached. The freezing-point depression of the roots, on the other hand, correlates very closely with the water content of the soil, in which they are growing, and with the concentration of the soil solution. Similar results were obtained when the freezing-point depressions of rye growing in soil of varying water content to which salts had been added, were determined. This is shown in Table XIII.

A similar set of experiments with corn plants, one foot in height, also was run. The results obtained appear in Table XVII.

The above data, as well as many others not presented in this paper, show that the freezing-point lowerings of the roots of plants indicate the water content of the soil in which they are growing, inasmuch as they are altered by changes in the water content of the soil. Moreover, they strongly indicate that the concentration of the soil solution at the wilting

point of the soil under ordinary conditions of temperature, etc., is equal to or greater than that of the roots of the plants. Again, we have failed to obtain evidence in these and many other determinations that the freezing-point lowerings of the leaves of plants are greatly affected by the water content of the soil until the critical point is approached. We should not fail to note that all comparative determinations of the leaves were carried on at approximately the same hour of the day—between 8.00 and 9.00 a. m.

TABLE XVII

THE FREEZING-POINT LOWERINGS OF THE ROOTS AND LEAVES OF THE CORN PLANT AS INDICATORS OF THE WATER CONTENT OF SOILS

Condition of Plants	Soil			Roots		Tops		
	Freezing-point-depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point-depression	Osmotic pressure atmospheres	Freezing-point-depression	Osmotic pressure atmospheres	Water content per cent
Excellent	0.070	0.844	16.22	.393	4.740	.507	6.115	87.90
Good	0.320	3.857	5.21	.470	5.664	.500	6.025	88.10
Signs of wilting	1.000	12.040	3.23	.463	5.603	.473	5.694	88.00
Badly wilted ..	*		2.13	.810	9.755	.635	7.650	86.00

* Did not freeze.

Field Studies

Many determinations of the freezing-point lowerings of leaves and roots of different crops growing under field conditions have yielded much

TABLE XVIII

THE FREEZING-POINT LOWERINGS OF ROOTS AND TOPS OF PLANTS AS INDICATORS OF THE WATER CONTENT OF SOILS

Soil Class	Per cent of water in soil	Corn Leaves		Corn Roots	
		Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres
Sand	10.5	0.461	5.553	.254	3.064
Sandy loam	21.1	0.457	5.513	.227	2.742
Loam	22.6	0.482	5.804	.278	3.355
		Rye Leaves		Rye Roots	
Sand	12.2	1.220	14.68	.285	3.435
Sandy loam	20.5	1.235	14.86	.310	3.737
Loam	24.2	1.264	15.20	.315	3.797

information with respect to the moisture relations of soils and the plants growing therein, precautions having been taken to obviate local differences as far as possible. We have shown that the variations in concen-

tration of the solution of all classes of soils are not great at or near the point of saturation. Such being the case, the freezing-point lowerings

TABLE XIX

VARIATIONS IN THE MOISTURE CONTENT OF SOILS AS MEASURED BY THE CHANGE IN THE FREEZING-POINT DEPRESSION OF THE ROOTS AND TOPS OF PLANTS GROWING IN THEM

Soil Class	Per cent of water in soil	Corn Tops		Corn Roots	
		Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres
Sand	12.50	.475	5.724	.254	3.064
	3.04	.695	8.372	.745	8.973
Sandy loam	20.00	.520	6.266	.325	3.917
	7.50	.785	9.454	.750	9.033
Muck	49.00	.601	7.239	.455	5.302
	40.50	.655	7.890	.610	7.349

of the roots and leaves of a given crop growing under such conditions should not vary greatly, unless the composition of the soil solution is an important factor.

TABLE XX

FREEZING-POINT LOWERINGS OF ROOTS AND LEAVES OF DIFFERENT CROPS GROWING UNDER SIMILAR FIELD CONDITIONS

MUCK SOIL

Crop	Soil			Tops			Roots	
	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres	Freezing-point depression	Osmotic pressure atmospheres	Water content per cent	Freezing-point depression	Osmotic pressure atmospheres
Canada Pea	43.30	.320	3.857	0.670	8.071	89.20	0.475	5.724
Corn	43.30	.320	3.857	0.430	5.182	87.80	0.412	4.961
Oats	43.30	.320	3.857	0.780	9.394	89.20	0.450	5.423
Wheat	43.30	.320	3.857	0.860	10.360	87.00	0.410	4.941
Barley	43.30	.320	3.857	0.800	9.635	87.30	0.390	4.700
Rye	43.30	.320	3.857	0.820	9.875	86.90	0.500	6.025
SANDY LOAM SOIL								
Canada Pea	6.22	.718	8.648	1.090	13.20	81.50	0.842	10.14
Corn	6.22	.718	8.648	0.965	11.62	82.04	0.932	11.23
Barley	6.22	.718	8.648	1.275	15.34	.....	1.005	12.10
SANDY SOIL								
Canada Pea	3.04	.290	3.496	0.735	8.85	88.20	0.735	8.850
Corn	3.04	.290	3.496	0.690	8.31	87.70	0.745	8.973

In Table XVIII are presented the results obtained with corn and rye taken from different classes of soil in a certain field on the college farm.

The above determinations show that the freezing-point lowerings of the leaves and roots of the same crop growing on different soil classes of high water content do not vary appreciably.

On the other hand, the concentration of the cell sap of the roots of the same crop—as measured by the freezing-point method—is greatly altered by appreciable changes in the water content of the soil, as the following results obtained from field studies with corn show.

Freezing-point lowerings of different crops seeded the same date on muck, sandy loam, and sandy soils, respectively, were determined. The results as set forth in Table XX bring out that the leaves of crops growing under adjacent field conditions differ markedly in concentration and the roots much less.

SUMMARY

Consistent determinations of the freezing-point depression of plant tissue may be obtained by inserting the thermometer directly into material triturated in the freezing tube.

Results with material repeatedly reduced to the point of solidification, repeatedly frozen at low temperatures, and material frozen at low temperatures and macerated or triturated were essentially the same as those obtained by direct freezing of the triturated material.

The freezing-point depression of sap expressed under great pressure from the aerial portion of the plants studied, previously frozen at low temperatures, was practically the same as that of the material frozen directly, but greater if the pressure is not so great. The freezing-point depressions of the juice expressed from roots frozen at low temperatures were not consistent.

It was found necessary to take samples of vegetable material for freezing-point work at the same time of day or else protect the plants from light and retard transpiration, since the freezing-point depression of the leaves was found to increase from morning till noon and decrease again in the evening. It appeared that this change was due to the products of photosynthesis, as well as to the moisture content of the material which decreased from morning till noon and increased again in the evening.

The concentration of the solution in which roots of Canada field pea and wheat are grown is indicated by the freezing-point lowerings of the root tissues, but on the other hand, only rather wide variations are indicated by the freezing-point lowerings of the tops.

Changes in the concentration of the soil solution induced by the addition of salts may be detected by determining the freezing-point lowerings of the roots of the plants growing therein. It was found that the tops of the plants are far less sensitive to changes in the concentration of the soil solution.

The moisture content of soils is closely correlated with the freezing-point lowerings of the roots of the plants in contact with them, due in part at least to changes in the concentration of the soil solution so induced, but again the tops of the plants studied, under both greenhouse and field conditions, prove to be far less sensitive to soil moisture changes, at least until the critical water content is approached.

The indications are that the soil solution and the root sap of plants approach each other in concentration at or near the critical (low) water content of the soil.

The freezing-point lowering of a given crop growing in widely different soils of high water content were found to differ but slightly. On the other hand, different crops growing on the same soil, under similar conditions vary appreciably in this respect.

The field studies show that crops may be subjected to sudden and very wide variations in the concentration of the soil solution during the growing season.

During the progress of the work herein reported many soil problems have suggested themselves. Some of these being studied are, the optimum concentration of the soil solution for crop production, the effects of different treatments upon the concentration of the soil solution at very low water contents, as measured by the freezing-point lowerings of the roots of plants, reasons for the adaptation of a given soil to the production of certain crops, as well as the changes, if any, induced in the concentration of the soil solution by crop production.

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THE SULFUR CONTENT OF SOME TYPICAL KANSAS SOILS, AND THE LOSS OF SULFUR DUE TO CULTIVATION[1]

By

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The importance of sulfur in the soil, both in relation to present crop production and permanent fertility, has recently become of great interest, at least, as judged by the number of publications on the subject. From a large number of soil samples collected in soil survey work by the chemical department of the Kansas Agricultural Experiment Station, a number of typical soils were selected and their sulfur content determined. These preliminary data seemed so significant that they are thought worthy of publication at this time. A series of soil samples is now being collected in connection with a study of the effect of prolonged production of alfalfa on soil fertility. The location of these samples has been carefully chosen with the main object in mind, but the samples will furnish very excellent material for further work on the sulfur problem.

That sulfur is an element essential to crop production has long been recognized by both botanists and agronomists. Sulfur is indispensable in the formation of plant proteins, and because of the intimate connection of protein compounds with life processes, it probably serves physiological functions in the formation of compounds which do not contain sulfur.

Growing plants get their sulfur directly from the sulfates of the soil. These originate in the action of ground water on sulfur-bearing inorganic compounds occurring naturally in the soil. The amount of sulfur from this source is small in most soils, although they act as a gathering agent for the sulfur compounds dissolved out of the air by rains. The amount of sulfur which reaches an acre of land in this way amounts to about 7.4 pounds per year, according to measurements made at Rothamsted (6). Hart and Peterson (7) estimate the amount for Wisconsin conditions to be about 7 pounds per year. The greater part of sulfur in most soils occurs in complex organic compounds (2).

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[1] Contribution from the Chemical Department, Kansas Agricultural Experiment Station. Received for publication October 9, 1916.

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Sulfur has not usually been considered a limiting element in crop production. The old method of determining sulfur in the ash of plants gave such small amounts, particularly in the grain, in comparison with the amount present in the soil, that the soil appeared to contain a sufficiency. Then also, sulfur was added in large amounts to old and worn soils in commercial fertilizers. One ton of acid phosphate contains about 80 to 120 pounds of sulfur, one ton of potassium sulfate about 360 pounds, one ton of ammonium sulfate about 400 pounds, and one ton of gypsum about 360 pounds.

By the old method of determining sulfur in the ash of plants, most of the sulfur was found in the leaves and stems of the plants, and very little in the grain. From this fact it was reasoned that if roughage was fed on the farm and the manure saved, the supply of sulfur would be conserved as an indirect result of keeping up the needed supply of organic matter. C. G. Hopkins (10) gives the following figures for sulfur in some of our principal grains:

Wheat, 50 bushels	0.1 lbs.
Wheat straw, 2½ tons	2.0 lbs.
Corn, 100 bushels	0.2 lbs.
Corn stover, 3 tons	5.8 lbs.
Oats, 100 bushels	0.5 lbs.
Oat straw, 2½ tons	3.0 lbs.

Such figures, obtained from carefully made ash analyses, permitted the inference that the supply of sulfur in the soil would easily take care of itself if the organic matter was conserved.

The recent methods of determining sulfur in organic material have shown that the ash method is erroneous, as large amounts of sulfur are lost in the ashing process. The figures obtained by several investigators, notably Hart and Peterson (7), show that the amount of sulfur crops, especially the grains, is much larger than was formerly supposed. On the basis of the results obtained by Hart and Peterson, the figures for sulfur in the above grains would be:

Wheat, 50 bushels	4.25 lbs.
Wheat straw, 2½ tons	6.20 lbs.
	10.45 lbs.
Corn, 100 bushels	8.50 lbs.
Corn stover, 3 tons	7.47 lbs.
	15.47 lbs.
Oats, 100 bushels	6.66 lbs.
Oat straw, 2½ tons	10.67 lbs.
	17.33 lbs.

Evidently the amount of sulfur in the grain in some cases is larger than in the rest of the plant, and in others almost as large. It means that if the soil supply of sulfur is not larger than that of some other essential element, notably phosphorus, sulfur may be a limiting element in crop production.

For determining sulfur in soil the fusion method as used by Hart and Peterson (7) was employed. These soils had previously been analysed for nitrogen, phosphorus, potassium, calcium, organic carbon, inorganic carbon, and acidity. All soils were sampled to three depths. The soil numbers are the ones used in the other determinations previously reported (15). The sample number is denoted by a whole number, and the different depths by decimals. Thus, 1010.1, 1010.2, 1010.3, denotes respectively, 0-7 inches, subsurface, 7-20 inches, and subsoil, 20-40 inches, of sample 1010.

Description of Soil Samples

1010. Oswego silt loam, Allen County. This was in native bluestem cropped as hay. The land lies farily level, and has not suffered from erosion.

1024. Oswego silt loam, Riley County. This land was also bluestem meadow cropped as hay. The surface is gently rolling and the sample was taken at the highest point. No erosion had taken place.

1031. Osage silty clay loam, Greenwood County. This land was in native bluestem cropped as hay. It consisted of second bottom soil, nearly level, with good drainage.

1032. This soil is the same as 1031, but was cropped continuously to corn for over thirty-five years.

1036. Summit silty clay loam, Russell County. This was cropped continuously to wheat for over thirty years. It lies in the semi-arid portion of the state.

1037. This soil was the same as 1036, but was in native buffalo grass used as pasture.

1050. Marshall silt loam, Brown County. This land was well farmed, and has been cropped to corn, oats, wheat, and clover.

1076. A brown loam, Harper County. The subsoil has a remarkably open texture and the chief crop has been wheat.

1039. Shelby silt loam, Shawnee County. Cropped to corn, oats, and wheat for over thirty-five years. This land is rolling.

1141. Summit silt loam, Shawnee County. It was in native bluestem, cropped as hay. Its topography is rolling.

1164. Reno loam, Reno County. This land is fairly level and has been in general crops, mainly wheat, for about thirty years.

These soils were selected because they are typical soils of that part of the state where they were taken, and they represent the different

portions of the state. Five of these samples were from fields in the native sod, and the other six from fields in cultivation for about thirty to forty years. The complete analyses of the virgin soils are found in Table II, and of the cultivated soils in Table III.

In some soils the sulfur content of the subsurface soil is somewhat larger than in the surface soil, and in some less. The average is practically the same. The same statement holds true for the cropped soils. The sulfur content of the subsoil of the virgin soils is in all cases less than that of the surface soils, but in the cropped soils the amount in the subsoil is practically the same as in the surface. The soils in virgin sod will average nearly the same amount of sulfur as found in some Iowa soils (2), while the cropped soils average much less. The average sulfur content of the cropped surface soils is 41.48 per cent less than the average sulfur content of the surface soils in virgin sod. In the subsurface the difference is 50.11 per cent, and in the subsoil it is 23.74 per cent. This means that the sulfur content of the cropped soils averages about 40 per cent less than the soils in native sod.

It is true that not all these soils were in pairs i. e., one cropped and one in virgin sod of the same type in close proximity. There are two such pairs, however, and the results are grouped in Table I. The figures in this table lead to the same conclusion as when all the soils were averaged. This substantially agrees with the findings of O. M. Shedd on some typical Kentucky soils (13).

TABLE I

PERCENTAGE OF SULFUR IN TWO PAIRS OF SOIL SAMPLES FROM RUSSELL AND GREENWOOD COUNTIES, KANSAS

Soil No.		Per cent S in Virgin Soil	Per cent S in Cropped Soil	Per cent Loss
Virgin	Cropped			
1031	1032	0.044	0.027	38.53
1037	1036	0.062	0.036	41.56

If the average content of nitrogen in the cropped soils is compared with that of the virgin soils, an average loss of 38.13 per cent is found in the surface soil, 28.66 per cent in the subsurface, and 35.55 per cent in the subsoil. The cropped surface soils have 38.44 per cent less carbon than the virgin surface soils. In the subsurface the difference is 35.32 per cent, and in the subsoil 32.94 per cent. The soil samples taken in pairs show a loss of 26.6 per cent of nitrogen, and 35.6 per cent of carbon for soils 1031 and 1032, and 30.5 per cent of nitrogen and 34.5 per cent of carbon for soils 1037 and 1036.

The above figures show that the loss of sulfur in cropped soils is greater than the loss of either nitrogen or carbon. This is more marked in the four soils taken in pairs close together than in the average of all the soils.

The average phosphorus content of the cropped soils compared with that of the virgin soils shows no such difference. In fact, the phosphorus content of the cropped soils averages a shade higher than that of the virgin soils. In potassium, the cropped soils are decidedly higher than the virgin soils. Whatever meaning this may have, the samples from the cropped soils were originally as good as those from the virgin soils, and possibly better. In this respect the results differ from those reported by Shedd (13).

TABLE II

PERCENTAGE AMOUNT OF ELEMENTS IN VIRGIN SOILS

No. of Soil	Sulfur	Nitrogen	Carbon	Phosphorus	Potassium	Calcium
1010.1	0.036	0.189	2.07	0.042	1.46	0.36
1010.2	0.043	0.124	1.52	0.040	1.40	0.53
1010.3	0.027	0.079	0.66	0.032	1.46	0.42
1024.1	0.048	0.249	3.06	0.045	1.75	0.66
1024.2	0.057	0.130	1.34	0.052	1.62	0.68
1024.3	0.042	0.069	0.61	0.069	2.00	1.01
1031.1	0.044	0.227	2.84	0.058	1.71	0.45
1031.2	.....	0.162	1.91	0.054	1.80	0.49
1031.3	0.039	0.105	1.06	0.051	1.65	0.50
1037.1	0.062	0.213	2.46	0.051	2.10	0.76
1141.1	0.040	0.300	2.71	0.033	1.66	0.56
1141.2	0.032	0.184	2.59	0.019	1.70	0.60
1141.3	0.027	0.107	1.05	0.022	1.80	1.75
Average:						
Surface	0.046	0.236	2.63	0.046	1.74	0.56
Subsurface	0.044	0.150	1.84	0.033	1.64	0.58
Subsoil	0.034	0.090	0.85	0.035	1.73	0.92

Most of the nitogen of the soil is in the organic matter . The nitrogen content of the subsurface soil is approximately two-thirds that of the surface soil, and of the subsoil, about one-third that of the surface. The same general relationship holds for the carbon content, which is an index of the amount of organic matter present. Sulfur does not show this general relationship. If most of the sulfur in ordinary soil is in organic combination, as is generally held by investigators (2), it must mean that the sulfur in the surface soil after undergoing sulfofication is leached into the lower strata in the form of sulfates. If this reasoning is correct, the subsoil has more sulfur in the form of sulfates than the surface.

Nitrogen is lost from the soil by being used by the crop. For every bushel of corn, one pound of nitrogen is taken from the soil, and nitrogen is also removed by oxidation and leaching of organic matter. In some virgin soils, as has been shown by several investigators, more nitrogen is lost as a result of mere cultivation than is taken up by the crop.

If the average sulfur content of the virgin soils is assumed to be 0.041 per cent, and that of the cultivated 0.025 per cent, for the three strata, the virgin soils contain 4920 pounds of sulfur per acre 40 inches deep, and the cultivated soils 3000 pounds, making a loss of 1920 pounds. On the basis of analyses made by Hart and Peterson (7), 50 bushels of wheat require nearly 10.5 pounds of sulfur, and 100 bushels of corn nearly 16 pounds. On one of the fields sampled in pairs corn had been produced continuously for thirty-five years, and on the other, wheat for about the same length of time. Assuming that corn has yielded 30 bushels

TABLE III

PERCENTAGE AMOUNT OF ELEMENTS IN CROPPED SOILS

No. of Soil	Sulfur	Nitrogen	Carbon	Phosphorus	Potassium	Calcium
1032.1	0.027	0.167	1.83	0.059	1.69	0.63
1036.1	0.036	0.148	1.61	0.063	1.89	0.80
1036.2	.....	0.091	0.82	0.056	1.95	0.83
1036.2	0.028	0.056	0.49	0.056	2.05	0.82
1050.1	0.028	0.232	2.77	0.049	2.07	0.68
1050.2	0.021	0.176	2.19	0.046	2.23	0.78
1050.3	0.024	0.078	0.64	0.049	2.18	0.80
1076.1	0.017	0.089	0.78	0.036	1.91	0.42
1076.2	0.019	0.082	0.75	0.042	1.89	0.41
1076.3	0.016	0.061	0.52	0.038	1.94	0.48
1139.1	0.026	0.147	1.69	0.027	1.39	0.40
1139.2	0.022	0.113	1.34	0.025	1.38	0.42
1139.3	0.029	0.047	0.67	0.015	1.42	0.51
1164.1	0.028	0.094	1.06	0.023	2.02	0.39
1164.2	0.026	0.075	0.86	0.030	1.88	0.41
1164.3	0.031	0.050	0.55	0.027	2.15	0.47
Average:						
Surface	0.027	0.146	1.62	0.043	1.83	0.55
Subsurface	0.022	0.107	1.19	0.040	1.87	0.57
Subsoil	0.026	0.058	0.57	0.035	1.95	0.62

per acre per year, and wheat 15 bushels per acre per year for 35 years, one acre produced 1050 bushels of corn, and the other 525 bushels of wheat. If all the corn stalks and wheat straw had been removed, which was not the case, these grains would have removed, as a maximum, in corn 168 pounds of sulfur, and in wheat 110 pounds of sulfur. These amounts do not in any way correspond to the large losses shown by the chemical analysis of the soil.

F. W. Clarke (4) states that the average per cent of sulfur of the earth's crust is 0.11, and that of phosphorus is the same. Hilgard (9) gives the following figures as representing the average amount present in the sixteen large rivers of the world.

PARTS PER MILLION		PARTS PER MILLION	
CaO	42.2	N_2O_5	3.8
CO_2	42.0	Cl	3.7
SiO_2	16.4	Al_2O_3	3.1
MgO	14.7	Fe_2O_3	2.8
SO_3	8.0	K_2O	2.4
Na_2O	7.1	NH_3	0.7
		P_2O_5	0.3

According to these figures, sulfur leaches from the earth's crust faster than any other element, as measured by the amounts present. Those elements which are present in larger amounts than sulfur in the river water occur in an enormous amount as compared with sulfur. The leaching of carbon is not serious from the standpoint of crop production, as plants obtain their carbon from the air.

E. J. Russell (12), classifies the ions present in the soil solution as those which tend to dissolve, and those which tend to be precipitated. The SO_3 belongs to the former class, while P_2O_5 belongs to the latter. This brings out one reason for the fact that sulfur is lost in large quantities from cultivated soils, while phosphorus does not show losses in these same soils. Figures given by Hall (5) show that the average amount of sulfur leached from the soil amounts to three times the amount added by rainfall. If it were not for this loss by leaching the amount added by rainfall would practically restore that removed by cropping.

The sulfur in complex organic combination undergoes a cycle of changes analogous to that of nitrogen. Bacteria causing decomposition liberate sulfur as hydrogen sulfide from the organic combination. If the conditions are favorable other organisms oxidize this to free sulfur and the sulfur to sulfuric acid. This acid combines with the bases of the soil, and may be valuable in converting the insoluble phosphates into soluble forms (11). Calcium carbonate applied to soils increases sulfofication (1). In this respect the process is analogous to nitrification. Under suitable conditions nitrification is most rapid in soils rich in organic matter. Similarly, the addition up to a certain point of organic matter to soils increases sulfofication.

Concluding Discussion

1. In a previous publication the large losses of nitrogen and organic matter from cultivated Kansas soils has been discussed (16). The preceding paragraphs show that the per cent loss of sulfur from cultivated soils is proportionately equal to that of organic matter. It has also been shown that the loss of sulfur due to the amount taken up by the crop is insignificant as compared with the total amount which has

disappeared from the soil. This means that the sulfofication has been in excess of the needs of the crop, and the sulfates produced have leached out of the ground.

2. As measured by crop requirements, sulfofication has been much more rapid than nitrification. This is shown by a comparison of the figures for samples 1031 and 1032 surface soil. If corn production of 1032 had been 30 bushels per acre for 35 years, and all the stalks returned, the amount of nitrogen removed in the grain would be 1050 pounds. Analysis of this soil in comparison with 1031, which was in native sod, showed a difference of 1200 pounds. That is, the crop production accounts for nearly all the nitrogen removed. The figures for sulfur show a loss of 332 pounds per acre in the surface soil. The amount of sulfur removed in the corn grain would amount to only 89.25 pounds. These figures are based only on the analysis of the surface soil. The differences would be greater if all the strata down to 40 inches were considered, as the amount of sulfur in the lower strata is nearly the same as that of the surface, while the nitrogen decreases very much.

3. It has been shown in the paper referred to (16), that the loss of organic matter is one of the most serious soil fertility problems. The data and discussion in the present paper, together with references to the work of others, show that the loss of sulfur may be a very important factor in the loss of crop producing power. C. G. Hopkins (10) has discussed how nitrification may account for a sufficient amount of nitric acid to liberate enough phosphorus for the needs of the crop. Sulfofication would be an additional factor in liberating phosphorus. Neither nitrification nor sulfofication will take place to a sufficient extent in soils depleted of their organic matter. The loss of organic matter means the loss of the potential chemical energy needed to make plant-food available.

4. That the addition of sulfur-bearing compounds to certain soils will increase production has been shown by a number of investigators, some of the most recent being Hart and Tottingham (8), and O. M. Shedd (14), who cited the work of others. In soil fertility investigations in Kansas made by L. E. Call (3), sulfur applied in the form of potassium sulfate has not increased the yield of alfalfa or other crops. The reason for this may possibly be that in spite of the large losses of sulfur in cultivated soils, sulfofication still takes place sufficiently rapidly for the needs of the crop. The large loss in excess of that used by the crop makes this inference plausible. However, these losses cannot go on indefinitely without affecting crop yields, unless the losses are made good in some way.

5. Whether or not sulfur is at present a limiting element in the production of crops on Kansas soils, it is apparent that the supply of

this essential element is closely related to the supply of organic matter. These analyses have shown that sulfur is depleted as rapidly as nitrogen and organic matter. Alfalfa is one of the important crops of the state. This requires a large amount of sulfur. Hart and Peterson give the percentage of sulfur in alfalfa hay as 0.287. Analyses of alfalfa at this station show that alfalfa contains on the average 0.25 per cent of phosphorus. Of the two elements it would seem that sulfur would become a limiting factor sooner than phosphorus, unless sulfur is made available more rapidly than phosphorus. This, however, would not affect the ultimate supply.

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NITROUS NITROGEN IN IRRIGATED SOILS[1]

By

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The amount of nitrous nitrogen in normal soils is usually conceded to be very small, but the actual amount present in the soil under irrigated field conditions has not, so far as the authors are aware, been determined. In connection with the investigations (2, 3) which have been carried on at the Utah Experiment Station during the past twelve years on the influence of irrigation water upon the movement and production of nitric nitrogen in irrigated soils, it was thought desirable to make a study of the nitrous nitrogen content of the soil. Therefore, such determinations were made throughout four years and it is the purpose of this article to discuss the results thus obtained. Briefly stated, the outline of the work was as follows[2]:

The field was divided into plots of 1-26 of an acre and each plot was divided with laterals and necessary devices for distributing and measuring the water applied. The field was divided into five equal sets of plots: the first set was left fallow, the second planted to alfalfa, the third to corn, the fourth to potatoes, and the fifth to oats. Each of these sets was further divided so that one plot in each set received a maximum, one a medium, one a minimum irrigation, and one was not irrigated. The first season the plots were sampled in the spring, then before and after each irrigation during the summer, and again in the fall; while during the last three years the samples were taken in the spring, midsummer, and fall, and analyzed for nitrous nitrogen and moisture. The irrigation and sampling were so arranged that the results from the cropped irrigated plots could be compared with the non-irrigated plot of the same series and also with the fallow plots receiving a corresponding amount of irrigation.

[1] Received for publication October 2, 1916.

[2] For a complete description of the experimental field, together with a description of the methods employed and the results for nitric nitrogen, the reader is referred to the publications noted above.

Method of Analysis

The soil solution was obtained by means of the Pasteur-Chamberlain filter. For rapid work a series of 24 Pasteur-Chamberlain filters were used as shown in Plate I. A weighed quantity of soil, 50 gm., was titurated in a mortar with 250 c.c. of cold distilled water. The water contained a few drops of chloroform for the purpose of inhibiting bacterial action. The soil was titurated for two minutes, allowed to stand fifteen minutes, and then filtered through the Pasteur-Chamberlain filter. Fifty c.c. of this solution were treated with 2 c.c. of the nitrite reagent and allowed to stand for 15 minutes. This solution was compared with a standard nitrite solution containing 0.01 mg. of nitrous nitrogen treated in the same manner. The nitrite reagent was prepared by mixing equal volumes of standard solutions of a naphthalamine acetate solution and sulfanilic acid (1). Moisture determinations were made on the soil and all the results calculated to dry bases.

During the first season the samples were taken to a depth of 10 feet in the spring (before irrigation and after irrigation), and in the fall. They are of especial interest in that they show the quantity of nitrous nitrogen contained in the soil at various depths, and also the immediate effect of irrigation water upon the quantity and distribution of the nitrous nitrogen of the soil. The results are given in Table I as pounds per acre[1] of nitrous nitrogen found in the soil.

The most striking point brought out by these data is the uniformly low nitrous nitrogen content of all the soil. The nitrous nitrogen is about evenly distributed throughout the ten feet, the total quantity never exceeding 17 pounds per acre. There is, however, a slight seasonal fluctuation which is most pronounced in the case of the alfalfa-bearing soil. The application of irrigation to the alfalfa and oats decreases the nitrous nitrogen content of the surface foot of soil, while the corn and fallow plots both show an increase.

From the last results reported in the last column of Table I, it is clear that there is no uniform relationship between the nitric nitrogen content of a soil and its nitrous nitrogen content, for we find the nitrous nitrogen-nitric nitrogen ratio varying from as narrow as 1 to 3.03 to as wide as 1 to 80.

The work of the first season indicated that it was not necessary to determine the nitrous nitrogen content of the soil to a depth of more than one foot, since the nitrous nitrogen is uniformly low through the ten feet of soil. Therefore, during the years 1913, 1914, and 1915 a careful study of the nitrous nitrogen content of the first foot was made. In this work the samples were taken in spring—about the middle of April, midsummer, about the last of July, and in the fall—the last of October or the

[1] One acre-foot of soil is assumed to weigh 3,600,000 pounds.

first of November. The results, therefore, as reported in Table II represent the average of a number of determinations extending over three years and should represent very nearly the nitrous nitrogen content of

TABLE I

NITROUS NITROGEN CONTENT OF SOIL GROWING VARIOUS CROPS AS DETERMINED AT DIFFERENT SEASONS: RESULTS STATED AS NITROUS NITROGEN IN POUNDS PER ACRE

FALLOW SOIL

Period	1	2	3	4	5	6	7	8	9	10	Total	Total Nitric Nitrogen in 10 ft.	Ratio of Nitrous Nitrogen to Nitric Nitrogen
April	0.87	0.85	0.81	1.06	1.29	0.80	1.03	0.84	0.99	2.01	10.55	109.50	1:10.38
Bef. Irr. ..	0.84	0.49	0.20	0.38	0.32	0.37	0.62	0.17	0.38	0.21	3.98	82.23	1:20.60
Aft. Irr. ..	0.58	0.45	0.32	0.49	0.27	0.45	0.32	0.36	0.49	0.38	3.01	79.43	1:26.39
Sept.	1*	*	*	*	*	0.75	*	*	*	*	.....	.....	.....
Oct.	*	*	*	*	*	*	*	*	*	*	.....	.....	.....
ALFALFA SOIL													
Spr.	0.24	0.52	0.54	0.69	0.29	0.34	0.25	0.30	0.24	0.58	3.99	104.20	1:26.12
Bef. Irr. ..	0.86	0.87	0.43	0.42	0.49	0.41	0.37	0.48	0.37	0.68	5.38	33.07	1: 6.15
Aft. Irr. ..	0.48	0.58	0.36	0.63	0.62	0.56	0.50	0.43	0.61	0.60	5.37	41.80	1: 7.78
Sept.	*	0.28	0.13	0.17	*	*	*	0.10	0.19	0.23	1.10	87.10	1:79.18
Oct.	0.08	0.07	0.35	0.31	0.89	0.41	0.03	0.64	0.43	0.10	3.31	68.30	1:20.63
CORN SOIL													
Spr.	0.54	0.47	1.07	0.24	0.69	0.21	0.27	0.25	0.45	0.96	5.15	146.70	1:28.49
Bef. Irr. ..	0.55	1.55	0.64	0.43	0.64	0.61	0.55	0.50	0.48	0.48	6.43	134.40	1:20.90
Aft. Irr. ..	0.73	0.87	0.63	0.66	0.53	0.43	0.76	1.18	1.26	1.02	8.07	147.83	1:18.32
Sept.	0.10	0.70	0.15	1.61	0.17	0.08	0.26	0.25	0.19	0.14	3.02	94.30	1:31.22
Oct.	0.54	0.32	0.39	0.31	0.31	0.71	0.64	0.36	0.27	0.22	4.07	167.60	1:41.18
POTATO SOIL													
Spr.	1.12	1.84	0.84	0.61	1.22	0.81	0.78	0.73	0.48	0.49	8.92	92.90	1:10.42
Bef. Irr. ..	0.76	0.48	0.32	0.61	0.55	0.68	0.83	0.59	0.33	0.89	6.04	59.60	1: 9.87
Aft. Irr. ..	0.86	0.74	0.57	0.57	0.80	0.68	1.00	0.69	0.78	0.28	6.97	77.72	1:11.15
Sept.	1.78	4.73	1.33	0.45	2.34	2.02	1.69	2.59	*	*	16.93	51.30	1: 3.03
Oct.	*	*	*	*	0.44	*	*	*	*	*	0.44	52.10	1:118.3
OAT SOIL													
Spr.	0.61	0.60	0.32	0.42	0.56	0.44	0.47	0.37	0.45	0.42	4.66	155.18	1:33.30
Bef. Irr. ..	1.03	0.98	0.57	0.88	0.48	0.75	0.63	1.61	0.44	0.36	7.73	156.00	1:20.18
Aft. Irr. ..	1.15	0.87	1.24	1.48	0.49	0.56	0.99	0.72	0.73	0.57	8.80	198.83	1:22.60
Sept.	*	1.59	0.29	0.29	0.67	0.27	*	*	1.02	0.47	4.60	187.90	1:40.85
Oct.	0.64	0.32	*	1.71	*	0.07	0.32	*	0.02	1.20	4.28	567.30	1:13.25

1 "*" in this table indicates "none."

this soil when growing various crops and receiving differing quantities of water. Each result in Table II represents one determination each year for three years for each of the 5 plots, viz., alfalfa, oats, corn, potatoes, and fallow.

These results bear out the conclusions reached from the first season's work that the nitrous nitrogen content of the soil is very low and that it is higher in midsummer than in the fall. In the spring the plots receiving the greatest quantity of irrigation water are the highest in nitrous nitrogen, while in midsummer and fall, with one exception, the reverse is true, the nitrous nitrogen content being inversely proportional to the water applied. But from the results as a whole, we must conclude that the application of irrigation waters to soil varying in quantity from none to 37.5 inches per acre yearly has very little influence upon the nitrous nitrogen content of the soil.

TABLE II

NITROUS NITROGEN OF SOIL GROWING VARIOUS CROPS AND RECEIVING VARYING AMOUNTS OF IRRIGATION WATER
Stated as Pounds per Acre

Water Applied	No. Determinations in Avge.	Seasonal Date of Sampling			Average
		Spring	Summer	Fall	
None	15	0.3762	0.5785	0.4255	0.4600
15.0 in.	18	0.7588	0.4806	0.3906	0.5433
25.0 in.	15	0.3978	0.3132	0.1937	0.3016
37.5 in.	15	1.1880	0.1847	0.2243	0.5323
Average	..	0.6802	0.3892	0.3085	.....

The influence of the crop upon the nitrous nitrogen content of the soil is given in Table III.

TABLE III

NITROUS NITROGEN CONTENT OF SOIL GROWING VARIOUS CROPS
Stated as Pounds per Acre

Crop	No. Determinations in Avge.	Season			Average
		Spring	Summer	Fall	
Alfalfa	16	1.4705	0.7354	0.3496	0.8518
Oats	16	1.3589	None	0.2877	0.8233
Corn	16	0.1760	None	0.3503	0.2632
Potatoes	16	0.1616	0.6538	0.4137	0.3430
Fallow	16	0.2347	0.5544	0.1415	0.3102

The nitrous nitrogen content of the alfalfa soil is higher in the spring than that of soils growing other crops, probably because of the compact condition of the alfalfa soil. The quantity in the alfalfa soil decreases as the season progresses until there is only one-third as much found in the soil in the fall as in the spring.

The nitrous nitrogen content of the oat soil stands next to the alfalfa, but there is this important difference between the two sets of soils, while the alfalfa soil shows a nitrous nitrogen content of 0.74 pounds per acre in the summer, the oat land contained such minute quantities that it is

here reported as none. This must be an inherent difference, and not due to accident, for it was repeated in the sixteen determinations made extending over three years, while at this season of the year the alfalfa soil invariably showed measurable quantities of nitrous nitrogen present. Otherwise, the alfalfa and oat soils are similar, both showing less in the soil in fall than in spring.

The corn and the potato land contained less nitrous nitrogen than the alfalfa and the oat land, and further differed from these in that the quantity slightly increased as the season progressed. The fallow soil differed from all of the others in that the quantity of nitrous nitrogen reached its maximum in midsummer in place of fall and spring, as was the case with the other crops. This fact would point strongly to the conclusion that the rapid removal of the nitrous nitrogen by the growing plant in midsummer stimulates the action of the nitrifying organisms with the result that the nitrous nitrogen is oxidized as fast as found. The accumulation of the nitric nitrogen in the case of the fallow soil, on the other hand, has led to an accumulation of nitrous nitrogen in the soil.

TABLE IV

NITROUS NITROGEN FOUND IN SOIL GROWING VARIOUS CROPS
Stated as Pounds per Acre

Crop	No. Determinations in Avge.	Seasonal Date of Sampling			Average
		Spring	Summer	Fall	
Sugar Beets	4	0.2376	0.7200	0.6336	0.3144
Alfalfa	5	2.7210	1.1850	0.1707	1.3589
Oats	3	0.6754	1.0080	0.3395	0.6743
Oats and Alfalfa	3	6.7540	0.4357	0.2502	2.4800

These considerations led to the conclusion that possiby a soil richer in nitric nitrogen may contain much larger quantities of nitrous nitrogen than this soil, which has been cropped for nearly thirty years without the addition of manure. The result is that the nitrogen and organic content has become very low even for a soil of the arid regions. For these reasons determinations were made of the nitrous nitrogen in soil from one of the crop rotation series which was receiving small applications of manure. The rotation was as follows:

Alfalfa	3 years
Sugar beets	2 years
Oats	1 year
Oats and alfalfa	1 year

The soil and size of plots was the same as those used in the preceding work. Weighed fresh manure at the rate of 15 tons per acre was applied to the sugar beet plot in the fall and then thoroughly disked in the next spring before planting. Each plot received 25 inches of irrigation water

applied to the soil in five equal applications. Analyses were made for nitrous nitrogen during three seasons and the summarized results as reported in Table IV represent the average of several determinations.

While direct determinations of the nitric nitrogen content and nitrifying powers of soil from these plots showed them to be much higher than the ones previously considered, they are no higher in nitrous nitrogen; they thus show that in a good arable soil the nitrous nitrogen is either transformed into nitric nitrogen practically as fast as it is formed, or is carried to lower depths by the soil water, for there is no appreciable accumulation of nitrites in the soil.

Summary

The data reported in this article represent some eight hundred determinations of the nitrous nitrogen of a calcareous soil without crop, cropped to alfalfa, to oats and alfalfa, to oats, to potatoes, and to sugar beets. The water applied to the soil varied from none to 37.5 inches per acre yearly.

The nitrous nitrogen content of the soil was very low and was about evenly distributed throughout the ten feet. The total quantity found in an acre of soil to a depth of ten feet varied from a trace—too small to be determined by the method—up to 17 pounds per acre. No relationship was found to exist between the nitrous nitrogen and the nitric nitrogen content of the soil.

The application of irrigation water had no appreciable influence upon the nitrous nitrogen content of the soil.

There was a slight seasonal variation in the nitrous nitrogen content of the soil; in the alfalfa and the oat soils it was highest in the spring and decreased toward fall, while in the potato and the corn soils it was lowest in spring and increased toward fall. In the fallow the nitrous nitrogen was highest in midsummer.

The greatest quantity of nitrous nitrogen was found in the alfalfa soil during the spring, while the least was found under the oats during mid-summer.

The application of manure to the soil had no appreciable influence upon the nitrous nitrogen content of the soil.

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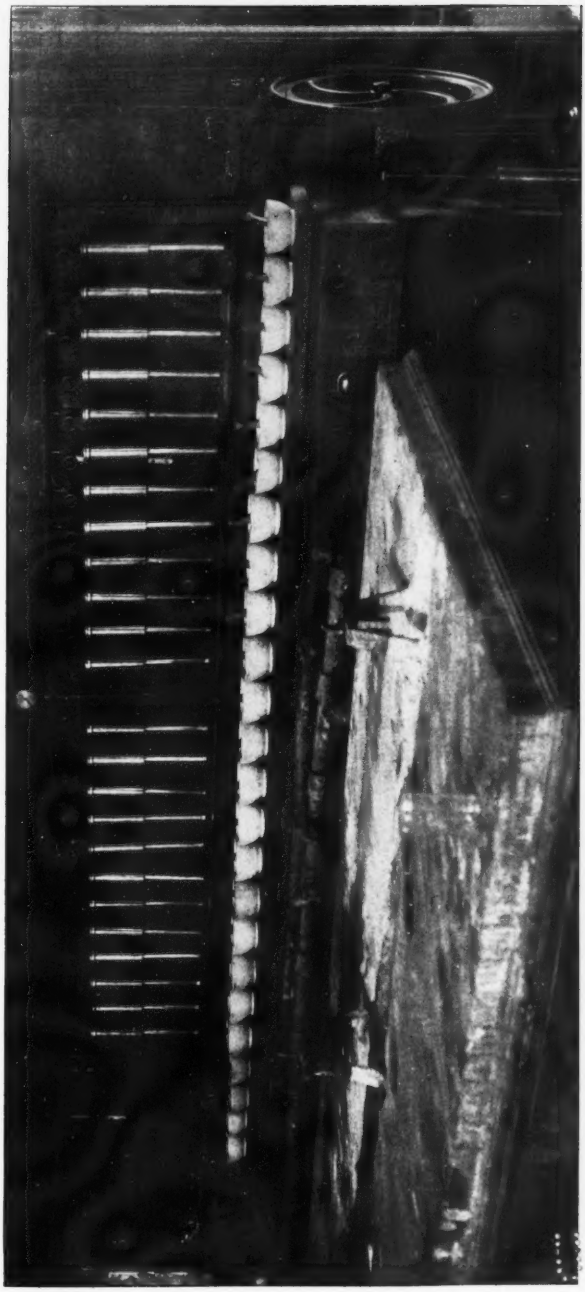
PLATE I

Pasteur-Chamberlain Filters used in analysis.



ASSIMILATION OF ORGANIC NITROGEN BY ZEA MAYS AND THE INFLUENCE OF BACILLUS SUBTILIS ON SUCH ASSIMILATION[1]

By

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STATEMENT OF PROBLEM

The aim of the work presented in this paper was first, to determine whether higher plants can utilize organic nitrogen directly without its being acted upon by microorganisms; second, to establish the relative importance of the compounds used; and third, to determine how the utilization of organic compounds by plants is affected by the action of a bacterium known to be able to decompose such compounds with the production of ammonia. The work embodies a series of experiments on the influence of different nitrogenous compounds, in sterile and inoculated cultures, upon the growth of seedlings of two varieties of Indian corn.

The problem was carried out under the direction of Professor J. B. Pollock of the Botany Department of the University of Michigan, and the author wishes here to make grateful acknowledgement to him for his assistance.

HISTORICAL INTRODUCTION

The discussion of soil fertility in modern times has centered upon the nitrogen problem. Nitrogen has long been known as one of the elements necessary for plant growth and is the one which must most continually be provided to keep up soil fertility, because it exists in such small quantities in the soil and is so easily removed by crops or by natural processes.

As long ago as 1835 Boussingault (5) showed that certain seeds contained as high as 4 to 7 per cent of nitrogen calculated on the dry weight basis. Later he (7) grew lupines, beans, and cresses in sand deprived of all nitrogen, and obtained about 1.3 per cent of nitrogen, showing probably a minimum requirement of that element in the plants. In the growth of soil fungi under nitrogen starvation conditions, Goddard (13) obtained from 1 to 2 per cent of nitrogen in the mycelium.

The growth of higher plants with an abundant supply of nitrogen shows that element to vary from 4.5 per cent in the leaves of red beets and in peas, 2.3 per cent in wheat grains, to 0.3 per cent in rye straw, ac-

[1] The data presented in this paper are from a thesis prepared in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan.

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cording to Jost (17). With soil fungi grown in a rich nitrogenous medium, Goddard (13) found about 5 per cent in the mycelium. The analyses of different species of mushrooms, as given by Atkinson (1), shows the amount of nitrogen to vary from 2 to 6 per cent.

The plant has three possible sources of nitrogen, namely, free nitrogen of the air and inorganic and organic compounds in the soil. The nitrogen problem has centered around first one and then another of these sources, and in later times about the action of bacteria in relation to all three sources.

The view was held from the time of Aristotle to about the end of the eighteenth century that humus was the source of all nourishment of plants, though the early Romans knew that the growing of leguminous crops on the fields in some way increased their fertility and they applied this knowledge to their argriculture. Following the discovery of the chemical elements the relation of these elements to the nutrition of plants became the subject of numerous investigations.

The view of Aristotle dominated until about 1840 even though Ingenhouze thought plants were able to absorb free nitrogen from the air. At this time the great German chemist Liebig (26) concluded that plants absorb all or most of their nitrogen in the form of ammonium compounds, that the nitrogen problem was purely chemical, and that free nitrogen could not be utilized. He held firmly to these conclusions throughout his life. Liebig's opinion probably hindered further progress at this time, because he was recognized as one of the greatest chemists and his views were generally accepted.

A new view was established about 1860, namely, that nitric acid or nitrates furnish an excellent, if not the most available source of nitrogen for the great majority of plants. This was given by Boussingault (6, 8, 9) at the conclusion of experiments carried on from 1835 to 1860.

The problem of the *Leguminosae* increasing the nitrogen was not explained by these views of Liebig and Boussingault, and numerous experiments were carried out, among which were those of Lawes, Gilbert and Pugh (23). These early investigations finally culminated in the experiments of Hellriegel and Wilfarth (15). They showed in the clearest way that microorganisms present in the soil are the cause of the formation of the nodules upon the roots of leguminous plants, and that when these nodules are present the assimilation of free nitrogen occurs.

These conclusions in regard to bacterial action in the nitrogen problem were followed in a short time by further proof of the rôle of bacteria in nitrogen transformation. It was Winogradsky (56) in his bacteriological studies, who ultimately cleared up the physiology of the nitro-bacteria, and his work has the right to be considered as one of the most important discoveries in plant physiology. He presented conclusive evidence of the existence of two kinds of nitro-bacteria; one of which pro-

duced nitrites from ammonia, and the other nitrates from nitrites. This discovery gave a clearer understanding of the old views of Liebig and Boussingault, and showed how organic compounds can become the source of nitrogen after first being ammonified and nitrified. In this process organic nitrogen is changed to inorganic which then is available for direct assimilation either as ammonium compounds or nitrates. Winogradsky (57, 58, 59) also discovered the non-symbiotic nitrogen-fixing bacteria living in the soil and studied their characteristics.

Organic Compounds

The direct assimilation of organic nitrogenous compounds was a part of the old humus theory and was held until Liebig's chemical theory began to prevail [Meyen (31) 1838]. Experiments tending to prove direct assimilation of such compounds were first made in 1857 by Cameron (10), with positive results. About 10 years later Wolf and Knop (60) also did similar work. Baessler (2), Lawes and Gilbert (22), and Berthelot (3) also have done some valuable work along this line. Since in these early experiments the significance of bacteria was not understood and the necessity for pure cultures was not recognized, all these early results are open to question.

Recent work on the assimilation of organic nitrogenous compounds has taken into account the possible action of bacteria and various investigations have indicated that these compounds are available for plants, although both negative and positive results have been obtained for the same compounds by various investigators. Strictly sterile conditions must be observed in testing accurately whether these compounds are directly assimilable or must first be acted upon by microorganisms to be ammonified or nitrified, or whether when so acted upon, they are rendered less toxic or more fully utilizable. There is also to be considered the difference in availability of the same substance for different plant species.

Suzuki (52) found that yellow lupines, potatoes, wheat and *Halesia hispidum* produced more asparagin from urea than from ammonium salts, while barley did not; and that, unlike nitrates, urea gave rise to asparagin in etiolated shoots.

Pryanishnikov and Lyebyedyev (40) in 1897 carried out experiments in sterilized and non-sterilized media with hippuric acid, urea, leucin, asparagin and aspartic acid. They found that none of the substances tested approached calcium nitrate as an effective source of nitrogen either in the sterilized or the non-sterilized media; also, that sterilization in all cases reduced the availability of the nitrogen of the organic substances, in most cases no gain being obtained in sterilized media.

Nakamura (37) in making quantitative comparison of asparagin and ammonium succinate as sources of nitrogen for barley, onions and *Asper-*

gillus oryzae, found that, in the case of the phanerogams, fully 50 per cent more growth was made where asparagin was added to the nutrient media than where the other compound was used. This was also true in the case of the fungus.

In 1898 Lutz (30) carried out some very extensive experiments upon the assimilation of organic nitrogen. These experiments were performed under sterile conditions, and thus fermentation products were excluded and nitrogen fixation prevented. The plants were grown in sterilized sand. The species used were, *Cucurbita maxima, Zea mays, Cucumis prophetarum, Helianthus annuus, Ipomaea purpurea, Onicus benedictus,* and *Cucumis melo.*

Trimeihylamin, dimethylamin, monomethylamin, diethylamin, propylamin and butylamin were all assimilated by the plants without first being fermented in the soil. Allylamin and benzylamin were found to be unfavorable to the growth of phanerogamic plants. The phenol-animes were toxic and the hydramines and pyridin bases were not assimilated. Tetramethylammonium and tetraethylammonium were not assimilated by phanerogamic plants. Among the alkaloids he found that caffin and quinin were toxic and cocain, atropin and morphin were not available.

Thompson (54) concludes from his studies with oats and barley that urea and uric acid have the same value for the grasses as nitric nitrogen, urea being slightly better than uric acid. His results indicate, however, that hippuric acid is detrimental to plant growth.

Pfeffer (39) has found that many heterotrophic organisms either require a supply of peptone or other proteins or attain their maximum development only when thus supplied. Phanerogams and algae can also employ as more or less valuable sources of nitrogen various organic substances such as: urea, glycocoll, asparagin, leucin, tyrosin, guanin, uric acid, acetamid, but none is as favorable to growth as sodium nitrate. He has also found that hippuric acid is decomposed by plants into glycocoll and benzoic acid, the latter of which is useless. He believed that the parts of the plant where such decomposition occurs are probably the same as those in which proteins are synthesized. Pfeffer holds that under natural conditions phanerogams rarely absorb organic nitrogenous compounds.

Schulze (49) quotes the investigations of a number of experimenters on the assimilation of leucin and tyrosin by plants and describes experiments of his own with lupines, vetches and castor beans, which showed that these chemicals could be used as sources of nitrogen by phanerogams.

Sawa (41), from investigations to determine if urea had any action on phanerogams, concluded that urea exercised an injurious action since the control plants made twice the growth of those in the solutions containing urea, and the branches were more vigorous on the control plants.

Kawakita (18) in his experiments on the effect of guanidin on plants found that solutions containing 0.5 gm. of guanidin in 250 c.c. killed

young barley plants in 3 days and that solutions one-fourth as concentrated killed the plants in 2 weeks.

Molliard (33) studied the value of asparagin and urea because, as he says, the assimilation of these two substances has been reported by others with different results. He grew his plant under sterile conditions and concludes from his experiments that these two substances maintain a nutrient rôle for higher plants.

Lefevre (24) in a series of experiments conducted with plants grown without carbon dioxide, found that glycocoll, alanin, tyrosin, and leucin not only furnish nitrogen, but also furnish the carbon required.

Schreiner and Reed (44) in their extensive studies tried guanin, although it is only slightly soluble in water. They used it in amounts varying from 1 to 40 parts per million, and in all of these concentrations it had a slightly beneficial effect upon the growth of wheat plants.

Guanidin carbonate, however, when tested on wheat plants in distilled water showed a very strong toxicity. When this solution was treated with carbon black, not only was the toxic action counteracted but the plants gave a better growth than the check in distilled water.

In a later publication (45) the same authors in their experiments found guanidin carbonate even in solutions so dilute as one part per million sufficient to kill wheat seedlings. Guanin was not harmful. Their experiments showed further that for wheat seedlings leucin and asparagin are not at all toxic. Alanin and glycocoll were slightly injurious at higher concentrations. Cumarin was extremely poisonous.

Bierema (4) reported that formamid and acetamid were not readily assimilated, although the latter was capable of supplying both nitrogen and carbon. Guanidin carbonate alone was not actively assimilated, but was somewhat more readily taken up in the pressence of calcium lactate, sucrose and glycerol. Uric acid was completely converted into ammonium carbonate, but less readily into urea. Leucin and tyrosin, especially the first, were readily assimilated, ammonium acetate more readily, especially in the presence of dextrose, and ammonium butyrate was still more readily assimilated.

Molliard (34) in further researches upon the utilization of organic nitrogen by higher plants, grouped his investigations under three main heads: (a) the action of various organic nitrogenous substances on the development and production of green and dry matter; (b) the total nitrogenous content of plants thus grown, and (c) the formation of protein substances from the absorbed nitrogen.

The following substances were used in the culture media in the ratio of 1:1000 parts: urate of sodium, aspartic acid, asparagin (1:500), glycocoll, legumin, cyanide of sodium, amygalin, hydrocyanic acid, leucin, tyrosin, myronate of potassium and alanin. Of these substances the first nine were utilized by the plants as shown by the increase in green and

dry matter over similar plants grown as checks. This utilization was the greatest in the case of urate of sodium, and decreased in order named down to leucin. Tyronsin, myronate of potassium and alanin were toxic to the roots only. The amount of protein nitrogen found in seedlings grown in the presence of asparagin and glycocoll was about twice the total nitrogen of the ungerminated seeds.

Hutchinson and Miller (16) in their work conclude that, while peptone and certain other nitrogenous compounds may be taken up and to some extent utilized by plants, they are unable to furnish the whole of the nitrogen required, or at any rate, to supply it with sufficient rapidity. They further conclude that their results are not sufficiently numerous to make it possible to trace any connection between the assimilability or non-assimilability of nitrogenous compounds and their constitution. They found it impossible to adhere to their original intention of sterilizing the media, for, although sterile media were most suitable, their employment was prevented by the impossibility of sterilizing many of the most desirable substances without more or less decomposition. They grouped the compounds experimented with under five heads, namely: (a) *readily assimilated*—ammonium salts, acetamid, urea, barbituric acid (with calcium carbonate), alloxan, humates; (b) *assimilated*—formamid, glycin, a. aminopropionic acid, guanidin hydrochloride, cyanuric acid, oxamid, sodium asparatate, peptone; (c) *doubtful*—trimethylamin (contrary to the results of Lutz), papa-urazine, hexamethylenetetramin; (d) *not assimilated*—ethyl nitrate, propionitrile, hydroxylamin hydrochloride, methyl carbamate; (e) *toxic*—tetranitromethane. This grouping, they affirm, is applicable only when peas are used and, as the authors suggest, it is possible that other plants may be able to utilize some of the substances which with peas have given negative results. Glycocoll in one culture gave an increase and in another a decrease.

Kossowicz (21) in his studies upon the assimilation of guanin and guanidin by mould fungi, found of about 10 fungi experimented with that all were able to utilize guanin as a nitrogen source, also guanidin under the conditions favoring the formation of ammonia. It is of interest that these results are different from those with the higher plants.

Schreiner (43) in his researches found that when creatin and nitrates are present less nitrates are used by the plant, although a larger plant growth takes place. The plant absorbs the creatin and builds it into its tissues. The author states that, upon his rather extensive investigations, he is ready to formulate the theory that the degeneration products of protein are absorbed directly by the plant from the soil and that the plant uses these units for building up the complex proteins as far as it is possible to do so. Since the plant must spend much energy in the building up of nitrates into amido groups of protein molecules, it is reasonable to suppose that the unit part of the complex molecule, when pre-

sented to the plant, will be used by it in preference to expending labor on the nitrate. The use of these decomposition products gives a different point of view to the problems of soil fertility.

Skinner (50) has shown in his experiments that the action of creatinin and creatin on growth is very similar. They had a beneficial effect on the growth where nitrate nitrogen was lacking and where only small amounts of nitrate were present, but when large amounts of nitrates were present these compounds produced no effect.

Skinner and Beattie (51) report that in all the plants experimented with asparagin is beneficial to growth, even when nitrate is present, although to a lesser degree.

Schreiner and Skinner (46) report upon some of the nitrogenous soil constituents as follows: Guanin at a concentration of 40 parts per million showed an increase in growth of 5 per cent over that of the growth in a distilled water control, and good root development. Asparagin showed, both in cultures with and without other sources of nitrogen, a decidedly beneficial effect upon the growth of plants. Guanidin produced a very decided toxic influence on growth. Glycocoll (amido-acetic acid) in water solutions was found to be beneficial. Alanin, in lower concentrations, was beneficial to growth, although in concentrations as high as 500 parts per million it slightly injured the roots of wheat seedlings.

Dachnowski and Gormley (12) in studies on bog plants and transpiration, together with the effect of glycocoll, state that the glycocoll is in part undoubtedly the gycocoll absorbed and assimilated.

Schreiner and Skinner (47) in experimenting upon the action of methyl glycocoll and glycocoll, found that the first was harmful, and the latter beneficial to the growth of plants.

It will be seen from the above review of the work on this subject that there is a great deal of contradiction in results obtained by different workers.

Bacterial Action

The process of nitrification was first shown in 1877 to be dependent upon the presence of certain micoorganisms, by Schloesing and Müntz (42). In 1893 Müntz and Coudon (36) showed for the first time that ammonia production in the soil is due to bacteria. However, in 1862 Pasteur (38) was the first to prove that the formation of ammonia from urea was brought about by the action of mocroorganisms. Within the last twenty years the work of numerous investigators shows that ammonia production from organic nitrogen is a function of most of the soil bacteria. Among the soil bacteria with this capacity is *Bacillus subtilis.*

Miquel (32) shows in his numerous experiments the effect of some of the species of bacilli which play an important rôle in the ammonifying of urea and splitting of uric acid into urea and other compounds. In

his conclusion he suggests that this splitting may play some part in the availability of these substances for the growth of plants.

Löhnis (29) found that soil bacteria rapidly convert urea into ammonium carbonate, probably by the action of *Urobacillus Pasteurii*.

The experiments regarding the decomposition of uric acid by bacteria, carried on by Liebert (25) showed that by aerobic bacteria the acid was broken up into carbon dioxide, ammonia, and the intermediate products, allantoin, urea, and oxalic acid.

Lipman (27) has recently determined that *B. subtilis* changes about 19 per cent of nitrogen present into ammonia.

Kelly (19, 20) has recently made extensive studies upon the biochemical decomposition of nitrogenous substances and ammonification, using commercial products such as casein, dried blood, cottonseed meal and linseed meal. The results showed that the different materials were converted into ammonia at greatly different rates and amounts.

New Experiments

In view of the contradictory results found by different investigators on the assimilation of organic nitrogen and in view of the desirability of testing more species of plants for their capacity of assimilating organic compounds, it was considered worth while to undertake the study of this problem with *Mays* plants grown with their roots in media free from bacteria except such as were intentionally inoculated into the cultures. The bacterium chosen was *B. subtilis*. This choice was so made because it is one of the common and widely distributed soil bacteria and has been shown to be capable of ammonifying organic compounds.

Methods and Technique

It is of the greatest importance that sterile, bacteria-free cultures be employed in investigations of soil bacteria, and especially in the case of experiments relative to the availability of organic nitrogen, for only thus can nitrification and ammonification be certainly prevented.

It is first necessary to select a medium which may be kept absolutely sterile throughout the experiment and which will permit the plants to make an active growth during a long period. Three media suggest themselves, namely, sand, water, and agar. Cultures in which each of these was employed were experimented with, and the latter was found to be the best adapted to the work at hand.

The plants which were placed in the sand cultures made a very poor growth and seemed to show evidence of a toxic influence. Warington (55) claims that such a material is in several respects a very unnatural medium for plant growth and is generally unsuited for this kind of investigation. Furthermore, it has so low a water-holding capacity that a culture when saturated contains about 60 per cent of inert material. Because of the small amount of water, some means of supplying sterile water

during the growth must be devised and this adds to the danger of contamininating the culture with fungi and bacteria.

Water cultures have been found satisfactory for a great deal of work by physiologists, but they require frequent change for the best results, and this is impractical under sterile conditions. Some means of aeration may be used. However, this can be done only at intervals, for continuous aeration is not practical with large numbers of cultures. Combes (11) suggests a method of aeration at intervals, but it requires special culture jars not easily obtainable. A preliminary experiment showed the poor growth of *Zea* plants in non-aerated water cultures.

Of the three media mentioned, the agar seemed then to afford the best substratum for the growth of the plants, the chemical compounds containing the mineral matters necessary for plant growth being added to it, of course. Some of the advantages of this media are: it makes possible the most rigidly pure cultures; the transparency of the agar permits the roots to be at all times visible; contaminations are easily recognized; and it affords a good mechanical support to the plants. The medium requires no attention beyond the initial preparation, that is, if a sufficient amount of medium is used at the beginning it does not require to be restored or renewed, even during a long period. This greatly lessens the danger of contamination. A 1-per cent agar solution was employed. This contained relatively little inert material, and sufficient water to last several months. The roots of the plants grew largely on the outside of the jelly-like, agar mass, which, as it gradually shrank away from the walls of the vessel, allowed good aeration of the roots. Agar was first employed as a culture substratum for green plants by Harrison and Barlow (14), who made use of it in their experiments with *leguminosae*. In the culture flasks in the experiment here recorded many of the plants grew well until all the water in the medium had been absorbed and the agar was dried down to a very small mass around the roots.

The agar medium was used in all of the experiments herein described, after the first preliminary ones. The roots of the plants were grown under sterile or inoculated conditions and the upper part exposed to the air. In order successfully to secure these conditions, some suitable culture jars had to be provided. For this purpose Erlenmeyer flasks of Jena, Resistenz or Bohemian glass of 700 and 1000-c.c. capacity were used. These were nearly filled with agar medium and sterilized in the autoclave for 20 minutes at 12 to 15 pounds pressure. Following the methods of Hutchinson and Miller (16), Schulow (48) and Combes (11) cotton plugs were placed in the mouths of the flasks, each plug rolled around a glass tube about 1 cm. in diameter and 15 cm. in length, through which the young plant could grow. This tube was also plugged with cotton. When the top was reached by the plant, the tube was withdrawn and the cotton pressed about the plant. This method allowed free growth of the leaves in the air, and aeration as well as sterile condition of the roots,

where this was desired. Each culture flask was wrapped with black paper to exclude light from the roots.

The experiments described here were carried out with two varieties of corn, namely, *Zea Mays everta,* Sturtevant (pop corn); and *Zea Mays indentata,* Sturt. (dent corn).

The pop corn seedlings were all grown in the greenhouse the first year, but the dent corn seedlings the second year were grown in the large south windows (9 feet high and 12 feet wide) of the laboratories of the Science Building, because the new botanical greenhouses were not completed and the old one was unavailable. The air of the rooms was kept moist by sprinkling the floors and having large pans of water exposed in the room. The cultures were maintained for two to three months.

Two methods were employed for measuring the growth of the plants. During the growth at various intervals and at the completion of the experiment the leaves were measured, and with the dent corn the dry weight of both the tops and the roots was obtained. The measurements were made first from the seed to the top of the youngest leaf and to this was added the length of each leaf from the stalk to its tip. The complete measurement of the plant was recorded. The measurements at intervals during growth did not reveal any special characteristics so they are not recorded in the data presented in this paper. The dry weights of the whole plant were determined after the removal of the remains of the seed. The roots were freed from the agar which remained about them by melting the agar in the autoclave and then washing the roots in boiling water. All of the substances occurring in the roots which are soluble in hot water were, of course, lost during this treatment. Each plant was then placed in a separate envelope and dried at a temperature of 80° C. to constant weight. These data of measurements and weights allow for accurate comparison with the checks which were grown in each series.

The results were recorded and studied in three different forms: by means of the tables compiled from the figures obtained and recorded later in this paper; by a comparison of photographs taken of the different sets grown at different times; and by graphs drawn for an easier and more ready comparison. Inoculated and sterile cultures were compared.

Twelve cultures were prepared in a set, each containing the same nitrogen source. Six of each set of 12 cultures were sterile and 6 were inoculated. One healthy seedling was planted in each flask. For the first series, the flasks were inoculated with 10 drops of soil water, prepared by shaking 5 gm. of soil with 50 c.c. of distilled water. In all of the other series where the flasks were inoculated, a pure culture of *B. subtilis* was used. A loop of bacteria was transferred with a sterile platinum wire loop from an agar slant to the warm liquid agar culture flask and thoroughly distributed through the medium by stirring with the sterile needle and shaking. In every case the inoculated flask showed a good growth of the bacteria.

Seed Sterilization

At the beginning of the work the seeds were sterilized by immersing in a water solution of mercuric chloride, 1:500, for 20 minutes. The seeds were first immersed in alcohol to remove any film of air. After the mercuric chloride treatment they were rinsed in sterile water to remove the sterilizing agent. This method was successful for the dent corn; but when the pop corn was so treated only a poor germination was obtained, and weak seedlings resulted from the few seeds that did germinate.

These results made it necessary to employ some other method for sterilizing pop corn, and following the suggestion of Lipman and Fowler (28), sulfuric acid (1.84 specific gravity) was tried. The best results were obtained by immersing the seeds for 4 minutes and then rinsing them in sterile water.

The sterilized seeds were placed on moist filter paper in sterile Petri dishes and allowed to germinate. In three or four days those that germinated well were transferred with sterile forceps to the surface of the agar medium in the flasks, and later the young shoots were directed into the glass tubes, which reached above the cotton stoppers. Germinating the seeds on agar was tried but the surface was too dry for the best results. Great care was used in selecting the seedlings to have them as nearly alike as possible, yet in spite of this precaution there was considerable difference in the rapidity of the growth during the first two weeks. Some that appeared healthy would not reach the tops of the tubes for a week or more after others which seemed equally as good. This difference is one of the greatest sources of error in the method used but its effect is minimized by the use of large numbers of plants.

Nutrient Solutions

The nutrient solution in these cultures was one which has been found to be most successful by Professor Pollock, after extensive experiments in his laboratory. The tribasic calcium phosphate was used instead of the acid phosphate to assure an alkaline medium. This has low solubility but by using an excess of the phosphate the solution was constantly kept supplied with a quantity sufficient for the growth of the plants. The amount of the different organic nitrogenous compounds to be used in the various solutions was determined upon the basis of furnishing in each solution the same amount of nitrogen that was present in the .004 M. solution of sodium nitrate used. Since peptone does not have a definite chemical formula, the nitrogen could not be accurately calculated, but it was estimated that 0.2 gm. of peptone in a liter of water would give the required amount of nitrogen.

A stock solution was used for the check and to this was added single nitrogenous substances in the preparation of the other media. The following is a list of the substances used in the stock solution, together with the

number of grams per liter of water of each substance used: calcium phosphate (tribasic) 1.240; magnesium sulfate 0.246; potassium chloride 0.298; ferric chloride 5 c.c. of .001 M. solution. This solution furnishes all of the elements necessary for the growth of green plants except nitrogen, and those obtained from water and carbon dioxide.

The other culture media were made up by adding each of the following substances to the stock solution (the number of grams of each used per liter of stock solution is indicated): sodium nitrate 0.340; urea 0.120; peptone 0.2; guanin 0.120; guanidin carbonate 0.180; benzamid 0.484; caffein 0.194; alanin 0.364; ammonium sulfate 0.264; asparagin 0.264; glycocoll 0.300; uric acid 0.168; diphenylamin 0.676; guanidin nitrate 0.122; hemoglobin 0.634; casein 0.459; linseed meal 1.120; cottonseed meal 1.090; malt 1.596; creatin 0.174.

With the exceptions of cottonseed meal, malt, peptone and linseed meal, all of the substances used in these nutrient solutions were chemically pure, and distilled water from the chemical laboratory was used in all cultures. The organic nitrogenous compounds employed were those prepared by C. A. F. Kahlbaum. The cottonseed meal and linseed meal were secured from a retail feed store, and the malt which was obtained from a brewery consisted of ground, sprouted barley grains.

Experiments

Pop Corn

Series I

The plants of this series were started the middle of October, 1914, and were harvested the middle of March, 1915. They were grown in the greenhouse in the following media: check consisting of the stock solution; and separate sets of media compound of stock solution to which sodium nitrate, urea, peptone, guanin and guanidin carbonate were respectively added. Half of the flasks were kept sterile while the other half were inoculated with soil water, as has been described above.

Soil water was not again used for inoculation because of bad results. The addition of this mixed culture of bacteria and fungi from the soil included some parasitic forms, which were detrimental to the plants. Therefore, in the succeeding series a pure culture of *B. subtilis* was used. The results of this series are incorporated in Tables I and II.

Series II

The cultures of Series II were started February, 1915, and harvested in June of the same year. They were grown in the greenhouse, and the same media were used as in Series I. The flasks which were here inoculated, had pure cultures of *B. subtilis* added, as has been described in the section on methods and technique. The plants of this series made a better and more uniform growth than those of Series I. Some of the plants

TABLE I

SERIES I, POP CORN, STERILE CULTURES
(October, 1914—March, 1915)

Culture	Number of plants	Total length of leaves cm.	Average length of leaves cm.	Per cent of average length of leaves of check
Check	4	255 200 150 145	187.50	100.0
Sodium Nitrate	4	425 365 335 245	342.50	182.6
Urea	3	300 290 165	251.70	134.2
Peptone	4	335 315 225 220	273.75	146.0
Guanin	2	180 115	147.50	78.6
Guanidin Carbonate*..				

* Plants small and died within a few days.

TABLE II

SERIES I, POP CORN, INOCULATED WITH SOIL WATER
(October, 1914—March, 1915)

Culture	Number of plants	Total length of leaves cm.	Average length of leaves cm.	Per cent of average length of leaves of check
Check	5	320 275 250 190 170	241.00	100.0
Sodium Nitrate	4	280 260 230 220	247.50	127.6
Urea	4	575 375 330 300	395.00	163.9
Peptone	4	230 220 215 180	211.25	87.6
Guanin	3	320 290 280	303.00	125.8
Guanidin Carbonate*..				

* Plants small and died within a few days.

showed contaminations which were parasitic. These were discarded from the data, and this was done in all of the following series. The contaminations in the flasks may have occurred on the seeds, some bacteria or fungi having survived the seed sterilization, or they may have gained entrance at the time of planting the seeds, when it was necessary to open the flasks. The results obtained here are similar to those of Series I, and may be studied by referring to Tables III and IV.

TABLE III

SERIES II, POP CORN, STERILE CULTURES

(February-June, 1915)

Culture	Number of plants	Total length of leaves cm.	Average length of leaves cm.	Per cent of average length of leaves of check
Check	12	290 285 275 265 250 230 230 210 205 200 195 185	235	100.0
Sodium Nitrate	7	450 445 405 370 355 335 325	384	163.4
Urea	8	370 360 355 295 280 270 230 230	299	127.2
Peptone	10	325 305 290 275 250 230 230 200	253	107.6
Guanin* ..... Guanidin Carbonate*..				

* Plants small, no roots, and soon died.

Series III

The plants in Series III were started the last of June, 1915, and harvested in about 8 weeks, the growth being very rapid during the long days and intense heat of the summer months. The greenhouse had rather poor means of ventilation and the glass was not painted so that the tem-

perature often reached 52° C., but the plants survived and made fairly good growth. The following nitrogen compounds were used in this set in addition to the check: sodium nitrate, urea, and peptone. The results are similar to those of the preceding series, and are recorded in Tables V and VI.

TABLE IV

SERIES II, POP CORN, INOCULATED WITH *B. SUBTILIS*

(February-June, 1915)

Culture	Number of plants	Total length of leaves cm.	Average length of leaves cm.	Per cent of average length of leaves of check
Check	11	365	246	100.0
		320		
		315		
		290		
		230		
		225		
		225		
		220		
		190		
		165		
		155		
Sodium Nitrate	7	610	407	165.4
		530		
		400		
		400		
		310		
		300		
		300		
Urea	11	400	370	150.4
		390		
		380		
		375		
		370		
		370		
		370		
		365		
		360		
		355		
		335		
Peptone	10	440	330	134.1
		420		
		360		
		320		
		315		
		315		
		310		
		305		
		265		
		250		
Guanin*				
Guanidin Carbonate*..				

* Plants small, no roots, and soon died.

The study of the data upon these first three series revealed very similar results in all. A summary of these results is shown in Table VII and figure 1. Because of the large number of plants grown, some definite conclusions may be drawn from the experiments, which will be stated later.

Series IV

The plants of this series were grown at the same time as, and under similar conditions to those of Series III, except that water cultures were used instead of agar cultures. There was no provision made for aeration.

TABLE V
SERIES III, POP CORN, STERILE CULTURES
(June-August, 1915)

Culture	Number of plants	Total length of leaves cm.	Average length of leaves cm.	Per cent of average length of leaves of check
Check	5	290 210 190 180 180	210	100.0
Sodium Nitrate	3	320 300 220	283	134.7
Urea	3	180 178 175	178	84.7
Peptone	5	420 370 300 245 230	313	149.0

In addition to the check, media containing the following nitrogen compounds were used: sodium nitrate, urea, peptone, benzamid, caffein, alanin, ammonium sulfate, and asparagin. By consulting Tables VIII

TABLE VI
SERIES III, POP CORN, INOCULATED WITH *B. SUBTILIS*
(June-August, 1915)

Culture	Number of plants	Total length of leaves cm.	Average length of leaves cm.	Per cent of average length of leaves of check
Check	6	320 260 250 230 195 190	224	100.0
Sodium Nitrate	4	430 410 310 170	340	151.7
Urea	4	585 430 325 280	405	180.8
Peptone	2	340 260	300	133.9

and IX and comparing the growth with that in the agar medium, it can readily be seen that the growth in these water cultures was exceedingly poor in all cases, and not nearly equal to that in the agar cultures. The

TABLE VII

SUMMARY OF DATA OF LENGTHS OF LEAVES OF 110 POP CORN PLANTS GROWN IN DIFFERENT CULTURE MEDIA, UNDER STERILE CONDITIONS AND INOCULATED WITH *B. SUBTILIS*, 1914-1915

Culture	Sterile Cultures		Inoc. *B. subtilis*	
	Average length cm.	Per cent of average length of check	Average length cm.	Per cent of average length of check
Check	227.7	100.0	243.9	100.0
Sodium Nitrate..	353.5	155.2	379.0	159.4
Urea	265.7	116.6	379.3	159.5
Peptone	272.3	119.6	325.0	133.2
Guanin*				
Guanidin Carbonate* ...				

* Toxic, no growth.

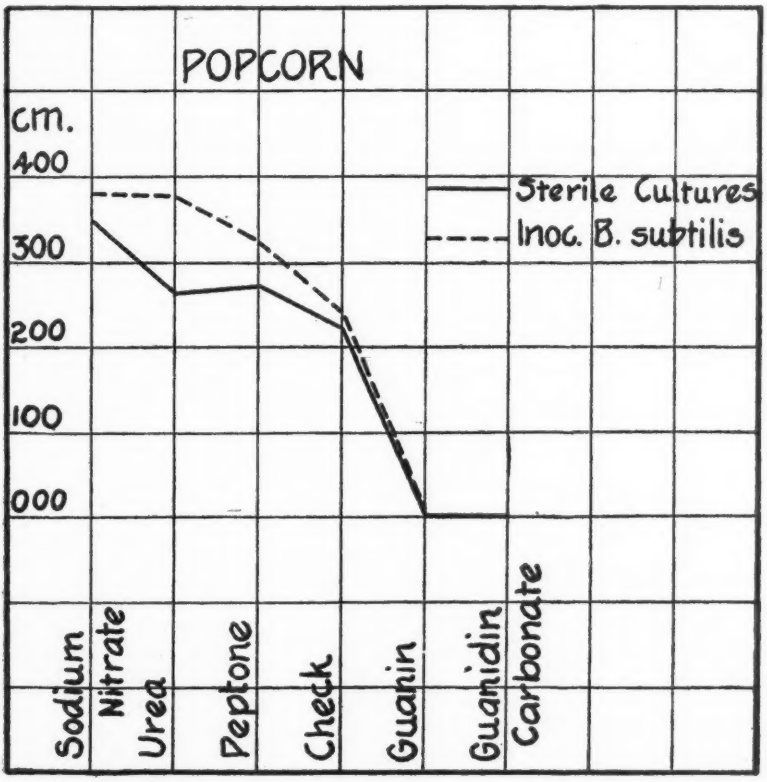

Fig. 1.—A summary of the results obtained from all of the pop corn plants grown in the experiment.

plants were weak and sickly. It was decided therefore, that unaerated water cultures were unsuitable for these experiments, and thereafter only agar cultures were used. The relative value of the nitrogenous substances in the water cultures was similar to that of these substances in the agar cultures, and may be used in connection with them.

TABLE VIII
SERIES IV, POP CORN, WATER CULTURES, STERILE CONDITIONS
(June-August, 1915)

Culture	Number of plants	Total length of leaves cm.	Average length of leaves cm.	Per cent of average length of leaves of check
Check	4	160	123.0	100.0
		120		
		110		
		100		
Sodium Nitrate	5	310	228.0	185.3
		240		
		240		
		190		
		160		
Urea	3	165	160.0	130.0
		160		
		155		
Peptone	4	230	170.0	138.0
		210		
		120		
		120		
Benzamid*				
Caffein	2	160	140.0	113.8
		120		
Alanin	4	140	127.5	103.6
		140		
		120		
		110		
Ammonium Sulfate	3	160	133.3	108.3
		120		
		120		
Asparagin	5	225	135.0	109.8
		120		
		115		
		110		
		110		

* Toxic, no growth.

Dent Corn

In the preceeding experiments some difficulty had been found in obtaining pop corn seedlings, and because of this fact and because it was advisable to try the effect of these substances upon another variety of the species, dent corn was used. Also other chemical substances were used as sources of nitrogen.

Series V

The plants of Series V were started in October, 1915, and harvested the following February. The plants were grown in the south window of one of the botanical laboratories. The light was not as good here as in

the greenhouse, but fairly uniform growth was obtained. The following nitrogen compounds were tested: sodium nitrate, urea, peptone, guanin, guanidin cabornate, guanidin nitrate, benzamid, caffein, alanin, ammonium sulfate, asparagin, glycocoll, uric acid, diphenylamine. In these, as in all the experiments, plants were grown in the stock solution as a check.

The results of these experiments are given in Tables X and XI. It is interesting to compare these results with those found in the growth of the pop corn plants. In general they are similar, but guanin which was toxic to pop corn was found to be quite beneficial to the dent corn seedlings.

TABLE IX

SERIES IV, POP CORN, WATER CULTURES, INOCULATED WITH *B. SUBTILIS*

(June-August, 1915)

Culture	Number of plants	Total length of leaves cm.	Average length of leaves cm.	Per cent of average length of leaves of check
Check	5	140	126	100.0
		135		
		135		
		120		
		100		
Sodium Nitrate	5	185	166	131.7
		175		
		170		
		170		
		150		
		150		
Urea	1	120	120	95.2
Peptone*				
Benzamid†				
Caffein	5	150	112	88.8
		115		
		110		
		95		
		90		
Alanin	2	130	120	95.2
		110		
Ammonium Sulfate	5	250	133	105.5
		160		
		90		
		85		
		80		
Asparagin	1	140	140	119.0

* No plants obtained. † Toxic, no growth.

Series VI

The plants of this series were started November, 1915, and the growth terminated during the next March. They were grown in a very poorly lighted window of one of the botanical laboratories, and consequently the growth was poor and very irregular. These facts must be considered in drawing any conclusion from the results of this series. The following nitrogenous substances were used: hemoglobin, casein, linseed meal, cottonseed meal, and malt. The results may be studied in Tables XII and XIII.

TABLE X

SERIES V, DENT CORN, STERILE CULTURES

(October, 1915—February, 1916)

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Check	5	285	219	0.42	0.30	100.0
		250		0.28		
		230		0.30		
		180		0.25		
		150		0.25		
Sodium Nitrate	4	410	321	1.08	0.71	236.6
		325		0.62		
		280		0.63		
		270		0.52		
Urea	4	320	182	0.75	0.33	110.0
		180		0.30		
		160		0.22		
		110		0.06		
Peptone	4	345	296	1.18	0.66	220.0
		345		0.60		
		260		0.50		
		235		0.35		
Guanin	5	404	351	1.24	1.10	366.6
		385		1.40		
		365		1.10		
		350		1.30		
		250		0.46		
Guanidin Carbonate .	4	165	138	0.35	0.25	83.3
		155		0.30		
		120		0.15		
		105		0.20		
Benzamid*						
Caffein	6	150	109	0.20	0.19	63.3
		145		0.31		
		135		0.21		
		110		0.20		
		80		0.13		
		25		0.10		
Alanin	6	420	300	1.90	.94	313.3
		365		1.28		
		320		1.05		
		270		0.51		
		225		0.47		
		215		0.44		
Ammonium Sulfate...	3	300	275	0.65	.54	180.0
		290		0.62		
		235		0.35		
Asparagin	5	365	319	1.40	.84	290.0
		335		0.94		
		315		0.71		
		310		0.70		
		270		0.45		
Glycocoll	5	365	281	1.14	.63	210.0
		325		0.70		
		245		0.52		
		240		0.40		
		230		0.40		
Uric Acid	6	280	256	0.48	.44	146.6
		270		0.47		
		265		0.57		
		265		0.38		
		260		0.45		
		200		0.31		

TABLE X—Continued

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Diphenylamin*						
Guanidin Nitrate	4	160	134	0.31	.24	80.0
		135		0.27		
		130		0.22		
		110		0.15		

* No growth, died within three days.

Series VII

This was one of the most successful sets grown during the year. The plants were started in January, 1916, and harvested in about three months. They were grown in a well lighted window of one of the laboratories of the Science Building, and the room was well heated. The following media were used: distilled water, check, sodium nitrate, urea, peptone, guanin, alanin, ammonium sulfate, asparagin, uric acid, hemoglobin, casein, linseed meal, cottonseed meal, malt, and creatin.

The plants of this series all made good growth and some interesting results were obtained, which may be readily seen by a study of Tables XIV and XV. The results of this and of the other series are discussed later in this paper.

Series VIII

The plants of this series were started in February, 1916, and harvested about two months later. They were grown in large test tubes. These plants had only about half the amount of medium that the plants of other series had, and consequently the growth had to be terminated at an earlier stage, but nevertheless, interesting results were obtained. The plants were grown in the new botanical greenhouse under very ideal conditions of light and heat, and a very good and uniform growth resulted. The following substances were used: check, sodium nitrate, urea, uric acid, casein, and cottonseed meal. The detailed results of this series are given in Tables XVI and XVII.

Discussion

The data presented in this thesis, comprise observations upon 614 *Zea Mays* plants grown until the water supply became exhausted in one or more of the culture flasks of a series. The conclusions are based upon the results of growth of these plants. This number does not include those plants which showed extreme toxic effects when young and made no further growth, nor those discarded because they were attacked by fungi. The large number of the plants employed makes it possible for us to draw certain fairly definite conclusions from the data secured.

The percentage of possible error in such work is a large one and must be taken into account in interpreting the result obtained. There are sev-

TABLE XI

SERIES V, DENT CORN, INOCULATED WITH *B. SUBTILIS*

(October, 1915—February, 1916)

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Check	6	215	181	0.30	0.25	100.0
		200		0.28		
		190		0.30		
		175		0.30		
		170		0.20		
		135		0.12		
Sodium Nitrate	4	340	265	0.65	0.40	160.0
		270		0.45		
		250		0.38		
		200		0.25		
Urea	5	345	231	0.60	0.30	156.0
		370		0.70		
		165		0.28		
		160		0.16		
		145		0.20		
Peptone	4	375	345	1.00	0.96	384.0
		350		0.93		
		330		0.70		
		325		1.20		
Guanin	6	400	311	1.07	0.73	292.0
		335		1.04		
		320		0.47		
		275		0.60		
		270		0.70		
		270		0.50		
Guanidin Carbonate..	4	210	171	0.44	0.31	124.0
		175		0.30		
		170		0.25		
		130		0.25		
Benzamid*						
Caffein	6	210	130	0.34	0.23	92.0
		170		0.23		
		140		0.22		
		115		0.22		
		80		0.20		
		55		0.15		
Alanin	6	430	300	1.20	0.88	352.0
		330		1.50		
		330		1.05		
		270		0.55		
		255		0.69		
		185		0.32		
Ammonium Sulfate ..	5	430	340	1.52	0.85	348.0
		345		0.97		
		325		0.75		
		300		0.65		
		300		0.54		
Asparagin	5	420	336	0.90	0.83	332.0
		400		1.34		
		300		0.69		
		285		0.68		
		275		0.57		
Glycocoll	5	325	240	0.82	0.56	224.0
		225		0.40		
		220		0.67		
		220		0.53		
		200		0.37		

TABLE XI—Continued

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Uric Acid	5	415	230	1.02	0.48	192.0
		320		0.50		
		215		0.32		
		170		0.29		
		170		0.27		
Diphenylamin*						
Guanidin Nitrate	3	150	135	0.26	0.22	88.0
		130		0.22		
		125		0.20		

* No growth, died within three days.

eral sources of error of which the most serious is the individual differences which occur between plants. No two individuals are exactly alike, as is shown, for instance, by the different growth vigor of different plants under identical external conditions. The degree of this error diminishes with increase in the number of plants. A second factor is the light relation. In the climate of southern Michigan, during the winter months on

TABLE XII
SERIES VI, DENT CORN, STERILE CULTURES
(November, 1915—March, 1916)

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Check	6	330	289	1.25	1.07	100.0
		320		1.52		
		300		1.22		
		300		0.90		
		285		1.00		
		220		0.55		
Hemoglobin	5	335	283	2.20	1.02	95.3
		335		0.55		
		280		1.34		
		255		0.60		
		210		0.40		
Casein	4	400	284	3.00	1.46	130.4
		255		1.00		
		250		0.50		
		230		1.35		
Linseed Meal	5	355	313	2.26	1.46	136.4
		330		1.70		
		315		1.05		
		285		1.30		
		280		1.00		
Cottonseed Meal	5	405	338	1.34	1.68	157.0
		370		2.20		
		350		1.65		
		310		1.67		
		255		1.55		
Malt	6	390	328	1.25	1.13	105.6
		380		0.95		
		335		2.35		
		300		0.85		
		300		0.75		
		265		0.65		

account of the shorter days, less intensity of the sunlight and the large proportion of the cloudy days, the light at the disposal of the plants is much less than during the summer months. As a result, the rate of growth is less than in summer. This fact must be taken into consideration when comparing the growth of series which were grown at different times of the year. Also, those plants which were grown in the laboratory windows did not receive as much light as those in the greenhouse, and those standing near the windows received more than plants farther back. This

TABLE XIII

SERIES VI, DENT CORN, INOCULATED WITH *B. SUBTILIS*

(November, 1915—March, 1916)

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Check	5	360	316	1.65	1.22	100.0
		335		1.15		
		325		1.40		
		310		0.70		
		255		1.40		
Hemoglobin	4	350	264	0.60	0.78	62.3
		260		1.30		
		260		0.85		
		185		0.35		
Casein	3	400	303	2.70	1.54	126.2
		300		1.42		
		210		0.50		
Linseed Meal	4	370	332	2.82	1.61	123.7
		365		1.30		
		315		0.90		
		280		2.19		
Cottonseed Meal	6	365	290	2.98	1.61	123.7
		310		1.50		
		300		1.70		
		280		1.17		
		255		1.25		
		230		1.05		
Malt	5	355	310	0.90	1.01	82.7
		345		1.45		
		340		1.25		
		320		0.90		
		235		0.56		

was controlled by shifting their positions during the period of growth. The diminishing of light causes a lessening of carbohydrate production and hence slower growth. This slow development may somewhat influence the assimilation of nitrogen. Another possible source of error is the wide temperature variations which occurred while some of the series were being grown. During vacations the heat in the building where the plants were grown was reduced and this caused a check in growth in Series V and VI from which the plants did not fully recover. These factors, then, which influence the percentage of error must be born in mind when making comparisons between different series. The large error due to differences in individual plants is well illustrated in the check solution of Series

TABLE XIV

SERIES VII, DENT CORN, STERILE CULTURES

(January-March, 1916)

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Distilled Water	3	275	220	0.93	0.62	47.3
		215		0.55		
		170		0.40		
Check	6	375	324	1.65	1.31	100.0
		360		1.20		
		345		1.30		
		330		1.20		
		305		1.41		
		230		1.15		
Sodium Nitrate	4	400	370	2.75	2.17	185.6
		385		2.60		
		345		1.90		
		290		1.42		
Urea	5	465	375	3.12	2.00	152.6
		430		2.40		
		390		1.73		
		320		1.50		
		270		1.27		
Peptone	5	435	353	2.47	1.78	135.8
		365		1.47		
		350		2.60		
		310		1.05		
		305		1.30		
Guanin	6	400	348	2.15	1.60	122.1
		360		1.90		
		360		1.90		
		350		1.20		
		345		1.42		
		275		1.05		
Alanin	4	335	291	2.00	1.50	114.5
		300		2.45		
		280		0.90		
		250		0.67		
Ammonium Sulfate...	6	495	425	2.70	2.45	187.0
		485		3.85		
		460		3.30		
		400		2.02		
		360		1.70		
		350		1.12		
Asparagin	6	560	523	5.10	3.89	296.9
		550		4.25		
		550		4.20		
		510		2.77		
		500		4.10		
		470		2.97		
Uric Acid	4	560	478	3.85	3.16	241.2
		520		3.35		
		435		2.47		
		400		2.98		
Hemoglobin	6	485	364	3.75	1.92	146.5
		425		2.60		
		365		1.42		
		325		1.20		
		300		1.52		
		285		1.02		

TABLE XIV—Continued

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Casein	6	490	422	3.65	2.68	204.5
		460		2.54		
		440		2.80		
		420		3.00		
		400		2.70		
		320		1.25		
Linseed Meal	5	415	327	2.75	1.57	112.2
		335		1.35		
		310		0.97		
		300		1.30		
		275		1.50		
Cottonseed Meal	6	410	321	2.90	1.83	139.6
		400		2.15		
		360		2.10		
		280		1.40		
		245		1.07		
		230		1.37		
Malt	6	330	301	1.25	1.05	80.1
		325		1.25		
		320		1.47		
		310		0.97		
		275		0.77		
		250		0.65		
Creatin	6	400	347	2.20	1.58	120.6
		360		1.45		
		355		1.65		
		350		1.40		
		345		1.45		
		275		1.37		

II. The largest plant measured 365 cm. and the smallest 155 cm., a difference of 210 cm. However, in all the checks of all the series, 48 plants in sterile cultures averaged 227.7 cm., and 49 plants in inoculated cultures averaged 221.6 cm. a difference of only 6.1 cm. With this number of plants the margin of error is very small.

The means for determining the amount of development of the plants in the various compounds used was, as has been stated above, by measurement of the length of the stalks and leaves, and by determining the dry weight. A comparison of the data obtained by the two methods shows that they are nearly parallel. The data show that in 19 cases the measurements and weights are, respectively, in the same relation in the sterile and the inoculated cultures; but in 5 cases they are reversed. This may be partly explained by the fact that the cultures in which these reverses occurred were checked in their growth, as has been explained. The leaves then did not develop well, but ears were formed which increased the weight. The weights probably serve a more definite and accurate basis for comparison than the measurements (cf. Tables X-XVII).

Since the problem was to determine the availability of various organic nitrogenous compounds for higher plants, the most logical means of discussion seems to be to take up each compound separately, explain the re-

TABLE XV

SERIES VII, DENT CORN, INOCULATED WITH *B. SUBTILIS*
(January-March, 1916)

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Distilled Water	2	220	180	0.72	0.50	34.5
		140		0.28		
Check	5	350	297	1.79	1.45	100.0
		330		1.68		
		325		1.65		
		265		1.20		
		215		0.95		
Sodium Nitrate	4	410	350	2.32	1.65	113.8
		370		2.80		
		320		0.80		
		300		0.70		
Urea	5	480	350	2.35	2.10	144.8
		465		3.95		
		405		1.67		
		355		1.65		
		225		0.70		
Peptone	6	490	435	3.45	2.35	162.0
		440		2.32		
		440		1.57		
		430		3.00		
		430		1.70		
		380		2.10		
Guanin	6	495	454	3.55	2.52	173.8
		490		3.10		
		470		2.25		
		465		2.35		
		420		1.92		
		385		1.97		
Alanin	6	400	333	2.97	2.10	144 8
		400		2.95		
		315		2.60		
		305		1.30		
		300		1.40		
		280		1.38		
Ammonium Sulfate...	6	510	462	3.45	3.15	217.2
		465		3.57		
		465		3.17		
		460		2.17		
		445		3.15		
		430		3.41		
Asparagin	4	610	561	4.69	4.24	292.4
		600		4.55		
		535		3.77		
		500		3.95		
Uric Acid	6	480	417	3.32	2.51	173.1
		475		2.52		
		460		3.55		
		400		3.12		
		390		1.60		
		300		0.97		
Hemoglobin	6	550	502	4.30	3.61	248.0
		540		3.15		
		535		4.27		
		510		3.80		
		505		3.65		
		375		2.49		

TABLE XV—Continued

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Casein	4	485	464	4.85	3.79	261.4
		470		3.42		
		450		3.57		
		450		3.35		
Linseed Meal	6	425	360	3.10	2.08	143.4
		375		1.65		
		350		1.95		
		350		1.32		
		330		2.85		
		330		1.60		
Cottonseed Meal	6	460	380	3.20	2.59	178.6
		400		2.82		
		370		2.20		
		370		2.12		
		360		2.52		
		315		2.70		
Malt	6	380	331	1.68	1.50	103.4
		375		1.55		
		370		1.75		
		360		1.92		
		270		1.10		
		230		1.02		
Creatin	6	390	340	1.60	1.44	99.3
		360		1.45		
		340		1.20		
		325		1.72		
		320		1.17		
		300		1.50		

sults obtained in the different cultures, and show the significance which they seem to reveal. Therefore, this procedure has been adopted.

Checks

The check solution was used in all the series. It contained all the chemical elements necessary for the growth of plants except nitrogen and those which the plant gets from the air. The growth in this solution was taken as the amount of growth allowed by the nitrogen supply in the seed. In the culture solutions containing nitrogen a growth markedly less than that of the check was interpreted as a toxic effect. A growth equal to that of the check was assumed to indicate that the nitrogen was not available. A growth markedly better than the check indicated that nitrogen in the form supplied was available. The plants grown in the check solution, toward the end of the period of growth, always showed the yellowing of the leaves, a characteristic effect of the lack of nitrogen.

The difference between the plants grown in the sterile cultures and in the inoculated ones is very slight. With the pop corn the plants inoculated in all cases were from 10 to 40 cm. better. Considering the measurement on length of all the check plants, those in the sterile cultures averaged 6 cm. per plant better than those in the inoculated.

Sodium Nitrate

A complete nutrient solution containing sodium nitrate was employed in all the series but one. Since the time of Boussingault (8) sodium nitrate has been considered one of the best, if not the best, source of nitrogen. It is in common use as a commercial fertilizer. In Series II, III, and VIII of the experiments it produced the best growth of all substances used. These were all grown in the greenhouse under favorable

TABLE XVI

SERIES VIII, DENT CORN, STERILE CULTURES

(February-April, 1916)

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Check	6	170	157.5	1.52	1.23	100.0
		170		1.25		
		160		1.10		
		155		1.30		
		150		1.25		
		140		1.00		
Sodium Nitrate	5	215	204.0	1.90	1.76	143.0
		215		1.80		
		205		1.72		
		200		1.60		
		185		1.80		
Urea	3	190	181.6	1.85	1.68	136.5
		190		1.70		
		165		1.50		
Uric Acid	6	200	179.0	1.80	1.50	126.8
		190		1.70		
		190		1.50		
		185		1.50		
		160		1.40		
		150		1.45		
Casein	6	205	185.0	1.65	1.41	114.6
		200		1.45		
		190		1.70		
		175		1.25		
		170		1.25		
		170		1.20		
Cottonseed Meal	5	160	148.0	1.28	1.14	92.6
		155		1.20		
		145		1.10		
		145		1.10		
		135		1.02		

conditions of temperature and light. In Series VIII, the experiment was discontinued after only two months had elapsed because with the small amount of medium used the water was exhausted at the end of that period.

Table VII and figure 1 show that in the growth of pop corn, sodium nitrate in sterile cultures was the best of the compounds tested as a source of nitrogen, while in the inoculated cultures urea equaled it in value. In the growth of dent corn the results in sterile cultures indicated that ammonium sulfate and asparagin are superior to sodium nitrate as a source

of nitrogen. In inoculated cultures, however, the following substances gave better results than the nitrate: asparagin, ammonium sulfate, peptone, guanin, uric acid, alanin, urea, hemoglobin, casein, linseed and cottonseed meals. The growth of the dent corn plants in the inoculated cultures of the nitrate was slightly poorer than in the sterile cultures, while

TABLE XVII

SERIES VIII, DENT CORN, INOCULATED WITH *B. SUBTILIS*

(February-April, 1916)

Culture	Number of Plants	Total lgth. of leaves cm.	Avg. length of leaves cm.	Dry weight gm.	Average dry weight gm.	Per cent of av. dry wgt. of check
Check	6	155	137.5	1.20	1.03	100.0
		155		1.15		
		140		1.15		
		130		0.80		
		125		0.92		
		120		0.95		
Sodium Nitrate	6	205	195.0	2.15	1.88	182.5
		205		1.82		
		205		1.85		
		195		1.80		
		190		2.10		
		170		1.55		
Urea	6	180	166.0	1.75	1.61	156.3
		175		1.88		
		175		1.35		
		170		1.90		
		160		1.90		
		135		0.90		
Uric Acid	5	190	174.0	1.80	1.57	152.4
		190		1.60		
		180		1.50		
		170		1.00		
		140		1.95		
Casein	6	215	185.0	1.92	1.56	151.4
		195		1.47		
		190		1.73		
		185		1.67		
		165		1.27		
		160		1.32		
Cottonseed Meal	6	180	161.0	1.45	1.18	114.5
		180		1.25		
		160		1.30		
		155		1.25		
		155		1.10		
		135		0.72		

in the pop corn plants the reverse was true, but the differences in both cases were within the range of error inherent in the method.

From these experiments it is clear that in all cases the growth of plants when furnished sodium nitrate was markedly better than when no nitrogen, except that in the seed, was present. The poorest showing for the nitrate was 113.6 per cent of the check in Table XV; the best was 236.6 per cent in Table X.

Urea

The plants grown in cultures containing urea as the source of nitrogen showed in the case of the pop corn a decidedly better development than those in the check solution. This indicates that the nitrogen of urea is available to some extent, but not sufficiently to make urea equal to sodium nitrate. However, in the inoculated cultures it proved equal to the nitrate as a source of nitrogen. This indicates that ammonification or some other transformation of urea is necessary for the best utilization and assimilation of that compound by pop corn plants. The weight of the dent corn plants in the sterile cultures showed urea to be about 50 per cent better than the check, though the leaf measurements were no greater

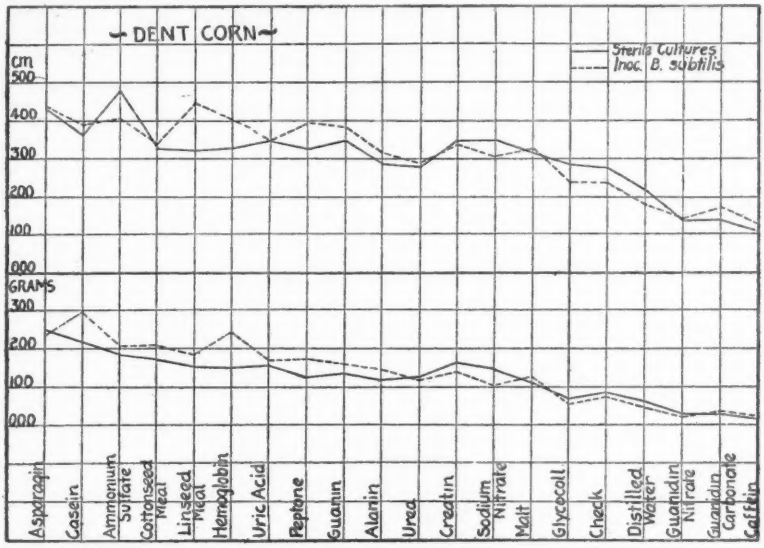

Fig. 2.—A summary of all the dent corn plants grown, showing both the dry weights and the measurements.

than those of the check. In the inoculated cultures both length and dry weight showed urea better than the inoculated check, and the dry weight showed even better growth than the dry weight of the plants in the inoculated cultures of sodium nitrate. A comparison of the sterile and the inoculated cultures, containing urea, shows no difference in the dry weight and the length of leaf is only slightly better in the inoculated cultures.

Urea was found unavailable by Pryanishnikov and Lyebyedyev (40), and toxic by Sawa (41). However, it has been reported beneficial by Hutchinson and Miller (16), Molliard (33), Suzuki (52) and Tompson (54). Takeuchi (53) has found that the enzyme, urease, which ammonifies urea, is not present in *Mays*. The corn itself then cannot ammonify urea.

Peptone

Peptone was utilized by *Mays* plants in both sterile and inoculated cultures. In the sterile cultures in both varieties of corn it was better than urea but did not quite equal the nitrate as a source of nitrogen. As with urea, the action of *B. subtilis* seemed to increase its availability for *Mays* plants. With pop corn, in the inoculated cultures, the growth was not equal to either that in the nitrate or urea but was considerably better than in the sterile cultures. With dent corn in sterile cultures the plants make a much better development than in the check but were not equal to those in the nitrate. The growth was 27 per cent better in the inoculated cultures than in the sterile. The plants with peptone were fifth in rank. Hutchinson and Miller (16) have found peptone a source of available nitrogen.

Guanin

Guanin was found to be exceedingly toxic to the pop corn plants in both sterile cultures and in those cultures which were inoculated with *B. subtilis*. These plants made practically no growth. In the cultures inoculated with soil water the growth was fair, but as there were only a few plants, no definite conclusions can be drawn. In the sterile cultures it was found to be about equal to the sodium nitrate for dent corn, and was better in the inoculated than in the sterile cultures.

The results show clearly that as a source of nitrogen the same chemical compound may have a value differing to a considerable degree for different varieties of a species. Guanin was toxic to pop corn and furnished available nitrogen to dent corn. Schreiner and Reed (44) and Schreiner and Skinner (46) have found guanin available to wheat seedlings.

Guanidin Carbonate

In the experiments guanidin carbonate was found to be exceedingly toxic to the pop corn plants used. Young seedlings made a very slight growth and died within a few days on culture media containing this substance. It was less toxic to dent corn but none of the cultures with this substance were as good as the check. Guanidin carbonate has also been found quite toxic to other plants by Kawakita (18), Schreiner and Reed (44, 45), Schreiner and Skinner (46), and Bierma (4).

Benzamid

The plants grown in a nutrient solution containing benzamid showed a decidedly toxic effect of this substance. They made a very feeble growth and died within 3 weeks. The action of *B. subtilis* did not alter the toxicity of this substance. Lutz (30) found that all compounds containing the benzin ring group were toxic to plants.

Caffein

The caffein nutrient solution with dent corn was more toxic than the guanidin carbonate, and much poorer than the solution used in the check culture. The leaves of the plant were small and pale in color. Those plants grown in the inoculated cultures were slightly better than those in the sterile cultures but the difference was not very marked. This compound has also been reported toxic by Lutz (30).

Glycocoll

Glycocoll was used as the source of nitrogen in only one series of experiments. The results of this series showed that it was favorable to the growth of *Mays* plants. It did not prove equal to sodium nitrate in the single series in which it was used. The growth was very little better in the sterile cultures. Its effect on other plants has been ascertained and found favorable by Schreiner and Skinner (46), Hutchinson and Miller (16), Dachnowski and Gormley (12), Lefevre (24), Molliard (34), and Schreiner and Reed (47).

Uric Acid

Thompson (54) has shown by his experiments that uric acid furnishes as good a source of nitrogen for oats as does urea and sodium nitrate. The results of the author's experiments with *Mays* are very similar. In the sterile cultures the growth of the dent corn was equal to that in the sodium nitrate both in length of leaves and in dry weight. Uric acid was better than urea as a source of nitrogen. There was only 1 cm. difference by measurement and .05 gm. by weight, between the averages of the sterile and the inoculated cultures in uric acid.

Diphenylamin

Diphenylamin was the most toxic substance used. When germinated seedlings were placed upon the agar medium containing this substance the roots turned brown and the plants died within 24 hours.

Alanin

The results of the experiments with alanin as a source of nitrogen, presented in Tables X, XI, XIV and XV, show it to be a good nitrogen source. In the sterile cultures the plants are nearly as good as those in the corresponding nitrate solution. The plants grown in the inoculated nitrate cultures are better than those of the sterile and better than the inoculated nitrate cultures. It is, therefore, a good source of nitrogen for *Mays*, although Schreiner and Skinner (46) found it slightly toxic to wheat seedlings and Molliard (34) reported it toxic to roots, while Lefevre (24) found it favorable as a nitrogen source.

Ammonium Sulfate

Ammonium sulfate was used in Series V and VII of the experiments. It has been known from the time of Liebig to be very readily assimilated by some plants. In the experiments here reported it was found to give a better growth of *Mays* than most of the other substances tried, and much better than sodium nitrate or urea. The measurements of the leaves show the sterile cultures to be slightly better while the weights reverse the ratio.

Asparagin

Asparagin is a substance found very widely distributed in plants, and the results obtained in these investigations show it to be an excellent source of nitrogen for *Mays*. The plants grown in this solution in sterile cultures are shown by measurements to be surpassed only by those in ammonium sulfate; by weights they are far better than any others. The growth in inoculated cultures is about equal to that in sterile. It has also been found readily assimilated in the experiments of Baessler (2), Molliard (33, 34), Nakamura (37), and Skinner and Beattie (51).

Guanidin Nitrate

The effect of guanidin nitrate upon *Mays* was about parallel to that of guanidin carbonate; approximately the same growth was obtained, the former showing about the same toxic reaction as the latter. There was a difference in weight of only .02 gm. between the plants in the sterile cultures inoculated with *B. subtilis*.

Hemoglobin

Hemoglobin is a complex animal protein, and it might be expected that, due to the molecular structure, the nitrogen would not be available for plants. The results show that in the sterile cultures it was slightly better than the check both by measurements and weights, but not as good as sodium nitrate. However, in the inoculated cultures the growth was about 25 per cent better than in the sterile, and much better than the inoculated check and nitrate cultures. The plants in this culture were among the best of all the cultures. A part of these plants supplied with hemoglobin were grown in very poor light and this may have had some detrimental influence, but even under such conditions they did exceptionally well.

Casein

Casein, like hemoglobin, is an animal protein, and might be thought to be unavailable for plant nutrition. Kelly (20) found that it may be readily ammonified by soil bacteria. The author's experiments show that in the sterile cultures it is favorable, about equal to sodium nitrate, and that in the inoculated cultures it is considerably better than the nitrate as a source of nitrogen. The inoculated cultures made a greater development than the sterile.

Linseed Meal

That such products as linseed meal and cottonseed meal might be used as a source of nitrogen was suggested by Kelly (19). The results here reported show that plants furnished with linseed meal make a slightly better growth in the sterile cultures than the check plants, but not equal to that of the plants having sodium nitrate. The inoculated cultures with linseed meal were decidedly better than the sterile and also better than the inoculated nitrate cultures.

Cottonseed Meal

The results with cottonseed meal were very similar to those with linseed meal. That is, in the sterile cultures the growth was only slightly better than the check but in the inoculated it is markedly better, and the plants here were among the best of all the cultures.

Malt

The plants grown in the solution to which malt had been added made approximately the same growth as those in the check in both the sterile and the inoculated cultures. This substance furnished practically no nitrogen, nor did the bacteria have any influence on the availability of the inoculated nitrate cultures.

Creatin

Creatin was used only in Series VII. This compound was of some value as a source of nitrogen, as indicated by the growth, which was somewhat better both in the sterile and in the inoculated cultures than the respective check cultures. There was little difference between them in growth in the sterile and in the inoculated cultures when the creatin was used as the source of nitrogen.

Chemical Groups

The inorganic nutrient salts, sodium nitrate and ammonium sulfate were both highly beneficial to plant growth as has already been stated, but they were excelled by some of the organic compounds. Among the organic compounds used were three purin derivatives. One, uric acid, was found available and decidedly beneficial, another guanin, was also found favorable to dent corn, while the third, caffein, containing three methyl groups, was quite toxic. The amids of the simple organic compounds are shown to contain nitrogen available for plant growth. Glycocoll and alanin are amids of acetic and propionic acids, respectively. Asparagin is a monamid of amido succinic acid and was one of the most favorable substances experimented with. Urea might be considered a diamido-carbonic acid, the simplest of all the organic acids. The albuminoid substances peptone, casein and hemoglobin were also available for plant nutrition.

The guanidin derivitives, guanidin carbonate, guanidin nitrate and creatin appeared to furnish the plant with no nitrogen. The first two were noticeably toxic in their action, while creatin seemed free from toxic properties.

Two compounds of the benzin ring group were used, benzamid and diphenylamin, the former with one, the latter with two benzin rings in the molecule. Both of these compounds were highly toxic to the *Mays* plants. The results with these two compounds are in accord with the work of Lutz (30) who has reported that benzylamin, diphenylamin, analin, and naphthylamin, members of the benzin series, were all toxic to the plants he employed.

Of the ground seeds, cottonseed meal and linseed meal contained available nitrogen for the plants, while malt was of no value as a source of nitrogen.

The results of this work and that of other investigators lead us to believe that some substances containing organic nitrogen may be used as a source of this element for plants in general. The fact that plants under experiment can absorb some of the substances, without first being broken down, indicates that this can take place with the plants in the fields since they grow in soils containing manure or other decaying vegetable and animal matter. Some of the substances then, in fertilizers, are directly assimilable by the plants and do not need to be ammonified and nitrified as is usually thought. Also, products probably occur in the intermediate stages of decomposition that may be directly utilized by plants. This is contrary to the general belief in agricultural practice that plants must be furnished with either ammonium compounds or nitrates. Nevertheless, most of the substances tried were utilized better or more rapidly when acted upon by *B. subtilis*. This is intelligible if *B. subtilis* causes ammonification of such substances, since ammonium sulfate was better than sodium nitrate.

Conclusions

The results of the investigations reported in this thesis warrant the following conclusions:

1. *Zea Mays* directly assimilates and uses the following organic nitrogenous compounds named in the order of their availability, asparagin, casein, cottonseed meal, hemoglobin, linseed meal, uric acid, peptone, guanin, alanin, urea, creatin, malt and glycocoll.

2. The following organic nitrogenous compounds are toxic to the growth of *Zea Mays:* guanidin carbonate, guanidin nitrate, diphenylamin, caffein, and benzamid. Guanin is toxic to pop corn but not to dent corn.

3. Eight organic substances which were directly available produced better growth when acted upon by *B. subtilis,* probably because of ammonification. These were peptone, guanin, alanin, linseed meal, cotton-

seed meal, casein, hemoglobin and urea. The last showed this effect only with pop corn.

4. The availability of the following substances was not increased by the action of *B. subtilis:* urea in the case of the dent corn, sodium nitrate, asparagin, ammonium sulfate, uric acid, malt, creatin, glycocoll, and those compounds which were toxic.

5. In the case of dent corn 6 substances were better than sodium nitrate; cottonseed meal, linseed meal, casein, hamoglobin, uric acid, and asparagin. The following, though available, were not better than sodium nitrate: urea, peptone, guanin, alanin, and creatin.

6. The different varieties of the same species of corn react differently with some nutrient substances. Guanin was toxic to pop corn but available to dent corn. Peptone was better utilized by dent corn than by pop corn.

7. The compounds of the benzin ring were found exceedingly toxic to the plants tried.

8. Ammonium sulfate is a far better source of nitrogen for dent corn than sodium nitrate, and is surpassed only by casein and asparagin, when tested by the dry weight, and only by asparagin when tested by length of leaves produced.

9. Generally, those organic compounds of high complexity in composition are better after ammonification, while those of a low degree of complexity are not improved by ammonification.

10. Very likely nitrification following ammonification would be detrimental, since sodium nitrate was not equal to ammonium sulfate for dent corn.

11. The method of measuring growth by length of leaves gave results very nearly parallel to those obtained by determining the dry weight, and is much simpler.

These conclusions apply to the two varieties of corn plants used. Only experiments on other species and varieties will show how they react to these substances.

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PLATE I

Fig. 1.—One of the 1000-c.c. culture flasks used in growing the corn plants; shows the rolled cotton plug and through it passing the glass tube, in which the plant grew through the cotton plug.

Fig. 2.—The 168 dent corn plants of Series VII. The flasks to the left of each number were sterile and to the right inoculated with *B. subtilis*. The various compounds used are : O, distilled water; I, check; II, sodium nitrate; III, urea; IV, peptone; V, guanin; IX, alanin; X, ammonium sulfate; XI, asparagin; XIII, uric acid; XVI, hemoglobin; XVII, casein; XVIII, linseed meal; XIX, cottonseed meal; XX, malt; XXI, creatin.

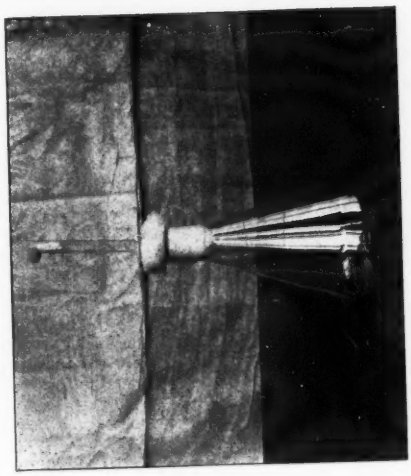

Fig. 1

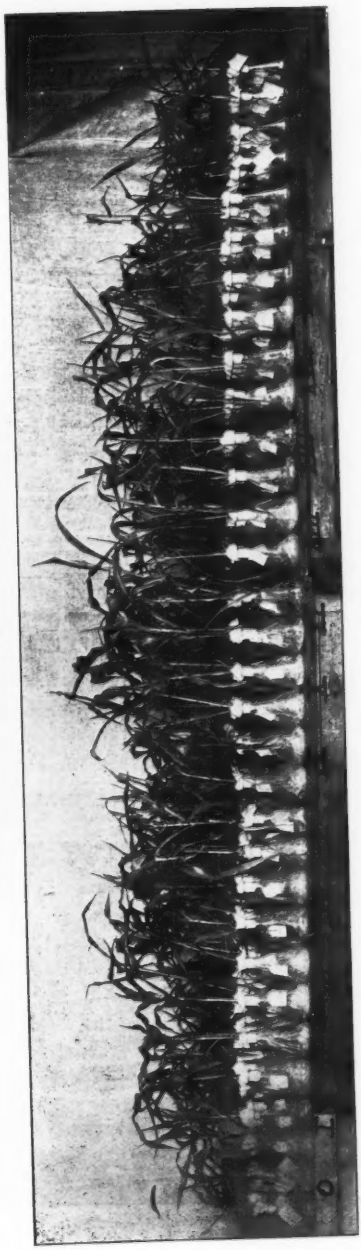

Fig. 2

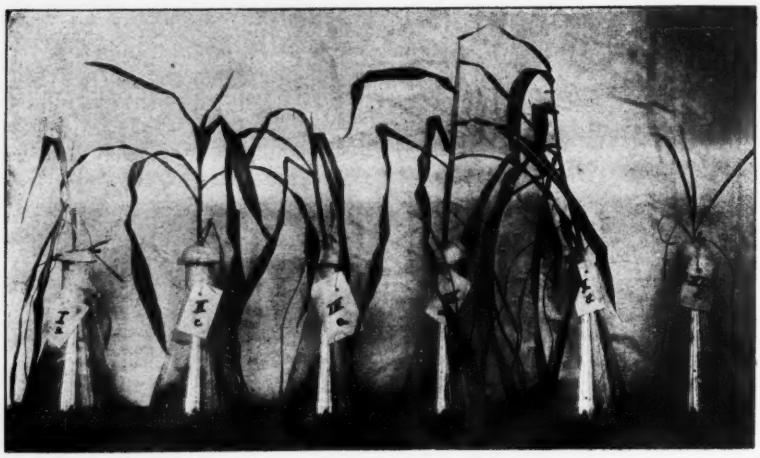

Fig. 1

Fig. 2

PLATE II

Fig. 1.—Dent corn plants grown under sterile conditions of Series V, with different forms of nitrogen added to the stock solution, namely, I, check; II, sodium nitrate; III, urea; IV, peptone; V, guanin; VI, guanidin carbonate.

Fig. 2.—Continuation of figure 1: IX, alanin; X, ammonium sulfate; XI, asparagin; XII, glycocoll; XIII, uric acid.